CHAPTER TWO

Intraflagellar Transport (IFT): Role in Ciliary Assembly, Resorption and Signalling

Lotte B. Pedersen* *and* Joel L. Rosenbaum†

Contents

Abstract

Cilia and flagella have attracted tremendous attention in recent years as research demonstrated crucial roles for these organelles in coordinating a number of physiologically and developmentally important signaling pathways, including the platelet-derived growth factor receptor (PDGFR) α, Sonic hedgehog, polycystin, and Wnt pathways. In addition, the realization that defective assembly or function of cilia can cause a plethora of diseases and developmental defects ("ciliopathies") has increased focus on the mechanisms by which

* Department of Biology, Section of Cell and Developmental Biology, University of Copenhagen, Universitetsparken 13, DK-2100 Copenhagen OE, Denmark
† Department of Molecular, Cellular, and Developmental Biology, Yale University, New Haven, Connecticut

Current Topics in Developmental Biology, Volume 85
ISSN 0070-2153, DOI: 10.1016/S0070-2153(08)00802-8

these antenna-like, microtubular structures assemble. Ciliogenesis is a complex, multistep process that is tightly coordinated with cell cycle progression and differentiation. The ciliary axoneme is extended from a modified centriole, the basal body, which migrates to and docks onto the apical plasma membrane early in ciliogenesis as cells enter growth arrest. The ciliary axoneme is elongated via intraflagellar transport (IFT), a bidirectional transport system that tracks along the polarized microtubules of the axoneme, and which is required for assembly of almost all cilia and flagella. Here, we provide an overview of ciliogenesis with particular emphasis on the molecular mechanisms and functions of IFT. In addition to a general, up-to-date description of IFT, we discuss mechanisms by which proteins are selectively targeted to the ciliary compartment, with special focus on the ciliary transition zone. Finally, we briefly review the role of IFT in cilia-mediated signaling, including how IFT is directly involved in moving signaling moieties into and out of the ciliary compartment.

1. Introduction

Cilia and flagella[1] are slender microtubule (MT)-based organelles with important motile and sensory functions. They consist of a highly organized MT-based axoneme that is extended from a modified centriole, the basal body, which anchors the axoneme in the cell. The ciliary axoneme projects out from the cell surface and is surrounded by a bilayer lipid membrane that is continuous with the plasma membrane of the cell body, but which contains a different complement of membrane receptors and ion channels (Fig. 2.1). Separating the two membrane compartments at the ciliary base is a region known as the ''ciliary necklace'' (Gilula and Satir, 1972), which is connected by fibers to the transition zone of the basal body (Fig. 2.1A, E, and H). It has been proposed that these transition zone fibers are part of a ''ciliary pore complex,'' which, by analogy to the nuclear pore, functions as a regulated gate of entry where ciliary precursors and intraflagellar transport (IFT) proteins accumulate prior to entering the ciliary compartment via IFT (Rosenbaum and Witman, 2002).

In general, two types of cilia exist: those that are motile and those that are nonmotile. However, whether motile or not, all types of cilia have important sensory functions that are critical for controlling a large number of cellular and developmental processes (Christensen *et al.*, 2007; Eggenschwiler and Anderson, 2007). Motile cilia and flagella typically contain an axoneme with nine outer doublet MTs, composed of A and B subfibers, which are held together by nexin links, as well as a central pair of MTs. These cilia are

1 Cilia and flagella are identical organelles, but for historical reasons, ''cilia'' is generally used when the organelle is present in multiple copies per cell while ''flagella'' is used when only one or two copies are present. Here, the terms will be used interchangeably.

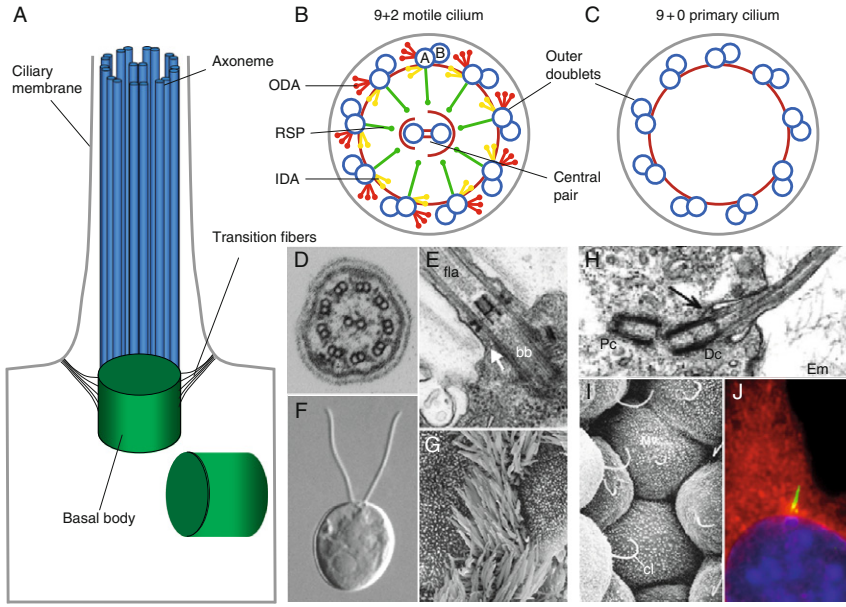

Figure 2.1 Diversity and structure of cilia and flagella. (A) Schematic showing the overall structure of a 9 + 2 cilium. (B) Schematic of a cross section of a 9 + 2 cilium highlighting the main axonemal components (ODA, outer dynein arm; IDA, inner dynein arm; RSP, radial spoke). (C) Schematic of a cross section of a 9 + 0 cilium that lacks motility-related structures. (D) TEM of a cross section of a *Chlamydomonas* flagellum (courtesy of Stefan Geimer, University of Bayreuth, Germany). (E) Longitudinal section of the base of a *Chlamydomonas* flagellum visualized by TEM. The arrow points to the flagellar base. *Abbreviations*: bb, basal body; fla, flagella. From Mitchell *et al.* (2005) with permission. (F) DIC of a *Chlamydomonas* cell with two 9 + 2 flagella. (G) Scanning EM of motile (9 + 2) tracheal cilia from a 4-week-old mouse (courtesy of Karl F. Lechtreck and George B. Witman, UMass Medical School, Worcester, MA). (H) Longitudinal section (TEM) of a primary cilium from a chicken chondrocyte showing the mother/distal centriole (Dc) and the daughter/proximal centriole (Pc). Arrow points to the transition fibers. *Abbreviation*: Em, extracellular matrix. Reprinted from Jensen *et al.* (2004) with permission. (I) SEM of ciliated renal epithelial cells from a kidney collecting tubule. *Abbreviations*: Ci, cilium; Mv, microvillus. From Kessel and Kardon (1979) with permission from Randy H. Kardon. (J) Immunofluorescence micrograph of a mouse NIH3T3 fibroblast stained with an antibody against detyrosinated α-tubulin to label the primary cilium (green) and with a $p150^{Glued}$ antibody that labels the ciliary base (red). The nucleus is stained with DAPI (blue). Courtesy of Jacob M. Schrøder, University of Copenhagen, Denmark, who also generated the completed figure.

hence designated "9 + 2" cilia. The axoneme of 9 + 2 cilia also contains accessory structures that are involved in motility, including outer and inner dynein arms, radial spokes, and central pair projections (Fig. 2.1A, B, and D). Sometimes 9 + 2 cilia are found in multiple copies per cell, for example, on the surface of epithelial cells lining the airways (Fig. 2.1G), oviducts, and brain ventricles, where the concerted action of the organelles mediates

transport of fluids and substances across the epithelial surface. Single-celled organisms like *Tetrahymena* and *Paramecium* also contain multiple motile 9 + 2 cilia on their surface, while mammalian sperm cells and the green alga *Chlamydomonas* contain one or two motile 9 + 2 flagella, respectively (Fig. 2.1F). Motile cilia on single-celled organisms play an important role in cell motility (Ibanez-Tallon *et al.*, 2003; Rosenbaum and Witman, 2002; Satir and Christensen, 2006).

While 9 + 2 cilia are the most commonly encountered type of motile cilia, there are examples of motile cilia that lack the central pair of MTs, for example, motile 9 + 0 cilia on the nodal cells of developing mammalian embryos, which rotate vigorously in a manner that generates a directional flow across the node required for establishment of the left–right asymmetry axis (Hirokawa *et al.*, 2006). In addition, motile cilia with four central MTs (9 + 4 cilia) have been identified on the notochordal plate of rabbit embryos, suggesting that there is some degree of variation in axonemal structure of motile cilia (Feistel and Blum, 2006).

Nonmotile (primary) cilia typically contain a 9 + 0 axoneme, but lack outer and inner dynein arms and other accessory structures involved in motility (Fig. 2.1C and H–J). One exception to this canonical 9 + 0 structure is the mature kinocilium of hair cells in the inner ear, which contains a 9 + 2 axoneme, but is considered to be nonmotile (Dabdoub and Kelley, 2005). Specialized sensory 9 + 0 cilia in the vertebrate olfactory organs as well as the outer segment of vertebrate photoreceptor rod and cone cells, which develops as an extension of a primary cilium, also display an atypical axonemal structure in that the distal region of the axoneme consists of extended singlet A MT subfibers, similar to neuronal sensory cilia of nematodes (Insinna and Besharse, 2008; Reese, 1965).

In addition to specialized primary cilia of sensory organs, primary 9 + 0 cilia are present in a single copy of almost all other cell types in vertebrate organisms when these cells are in growth arrest (see http://www.bowserlab.org/primarycilia/ciliumpage2.html for a comprehensive listing of cells with primary cilia), but despite a near ubiquitous presence in vertebrates and although they were discovered over a century ago, primary cilia were, until recently, considered vestigial organelles with no important function. However, a number of key discoveries during the past decade have dramatically changed this view and it is now well established that primary cilia are indispensable sensory organelles involved in coordinating and regulating a variety of crucial cellular and developmental processes (Christensen *et al.*, 2007; Davenport and Yoder, 2005; Eggenschwiler and Anderson, 2007; Pazour and Witman, 2003). The first two key discoveries leading to this "renaissance" of the primary cilium were the discovery of IFT in *Chlamydomonas* by Kozminski *et al.* (1993) and a study by Cole *et al.* (1998), demonstrating that *Chlamydomonas* IFT particle polypeptides are homologous to proteins required for sensory cilia biogenesis in nematodes (Perkins *et al.*, 1986). These two studies provided the foundation for Pazour and

coworkers who showed in 2000 that the mouse ortholog of one of the *Chlamydomonas* IFT particle polypeptides (IFT88/Tg737) is required for primary cilia formation in the kidney, and that failure to assemble these cilia leads to autosomal recessive polycystic kidney disease (Pazour *et al.*, 2000). Finally, work by Barr, Pazour, Yoder, and colleagues on the autosomal dominant kidney disease-associated gene products polycystin-1 and polycystin-2 revealed that these proteins are localized to sensory cilia in *Caenorhabditis elegans* (Barr and Sternberg, 1999) as well as kidney primary cilia in the mouse (Pazour *et al.*, 2002; Yoder *et al.*, 2002) providing a link between IFT and polycystin-mediated signaling. While these studies were instrumental in defining a role for IFT and primary cilia in sensory signaling and mammalian health and disease, they also spurred an immense increase in primary cilia research, eventually leading to the realization that primary cilia are indispensable sensory organelles involved in coordinating and regulating a variety of crucial cellular and developmental signaling pathways, including the polycystin, Sonic hedgehog (Shh), platelet-derived growth factor receptor (PDGFR) α, and Wnt signaling pathways (Christensen *et al.*, 2007; Davenport and Yoder, 2005; Eggenschwiler and Anderson, 2007; Pazour and Witman, 2003). Consequently, defects in the assembly or function of primary cilia can cause a plethora of diseases and developmental defects, which, along with disorders resulting from dysfunctional motile cilia, are now collectively known as ''ciliopathies.'' The ciliopathies, which include cystic kidney and liver diseases, retinal degeneration, polydactyly, left–right patterning defects, obesity, airway disease, hydrocephalus, male and female infertility, and Kartagener, Joubert, Bardet-Biedl, Senior-Løken, and Meckel-Gruber syndromes (MKS), have been described in several recent reviews (Badano *et al.*, 2006; Fliegauf *et al.*, 2007; Ibanez-Tallon *et al.*, 2003; Pan *et al.*, 2005; Pazour and Rosenbaum, 2002) and will not be dwelled upon extensively here. Another exciting topic in cilia research, namely the role of cilia in cell cycle regulation and cancer, has also recently been reviewed in detail elsewhere (Pan and Snell, 2007; Plotnikova *et al.*, 2008; Quarmby and Parker, 2005; Santos and Reiter, 2008) and will therefore not be covered extensively here.

In this review, we focus on the mechanisms by which cilia are assembled and maintained, with particular emphasis on the molecular mechanisms and functions of IFT (Kozminski *et al.*, 1993), a process required for the assembly and maintenance of almost all eukaryotic cilia and flagella (Rosenbaum and Witman, 2002). We first provide a general description of IFT, from its initial discovery to the identification and characterization of individual IFT motor subunits and IFT particle polypeptides. Next, we discuss the mechanisms by which proteins are selectively targeted to the ciliary compartment with particular emphasis on the function and composition of the ciliary transition zone. Finally, we briefly review the role of IFT in cilia-mediated signaling, including how IFT is directly involved in moving signaling moieties within the cilium and between the ciliary compartment and the cytoplasm.

2. Early Events in Ciliogenesis

The ciliary axoneme is assembled onto a basal body, which is derived from a centriole, and ciliogenesis is therefore tightly coupled to centriole duplication and maturation. In multiciliated, differentiated mammalian epithelial cells, centrioles are formed en masse from a fibrogranular assembly site and centriole production is usually independent of pre-existing centrioles, but related to differentiation. These centrioles undergo a complex and poorly understood maturation process and migrate to the ciliary assembly site, possibly by interacting with the actin cytoskeleton and proteins of the planar cell polarity (PCP) pathway (Dawe *et al.*, 2007a; Dirksen, 1991; Park *et al.*, 2008).

In proliferating cells that will form a primary cilium, ciliogenesis is tightly coupled to the cell cycle (Fig. 2.2). Ciliogenesis is initiated in G1 by the addition of Golgi-derived vesicles to the distal end of the mother centriole, and the axoneme begins to elongate within this membrane-bound compartment (Sorokin, 1962). As ciliogenesis proceeds, the axoneme continues to elongate from its distal end (Johnson and Rosenbaum, 1992) and the mother centriole-associated membrane vesicle grows by recruitment of additional Golgi-derived vesicles (Sorokin, 1962). Concomitant with docking of the mature mother centriole/basal body onto the ciliary assembly site at the apical end of the cell, the mother centriole-associated membrane vesicle fuses with the plasma membrane of the cell thereby forming a membrane sheath around the emerging ciliary axoneme that is separated from the plasma membrane of the cell by the ciliary necklace (Sorokin, 1962). Elongation of the membrane-bound axoneme is mediated by IFT (Rosenbaum and Witman, 2002). However, in a few cases, for example, in *Drosophila* sperm cells, assembly of the ciliary axoneme takes place in the cytoplasm by an IFT-independent mechanism (Han *et al.*, 2003), but this type of assembly will not be discussed further here.

New (daughter) centrioles are made from pre-existing (mother) centrioles in the S phase of the cell cycle and reach their mature length during late G2/M (Fig. 2.2; Azimzadeh and Bornens, 2007; Pan and Snell, 2007). When cells are in G1, the mother centriole is distinguished from the daughter centriole by the presence of two sets of nine appendages at its distal end termed distal and subdistal appendages, respectively. The subdistal appendages are thought to be required for anchoring of MTs at the mother centriole while the distal appendages are thought to be involved in the docking of the mother centriole/basal body to the apical cell membrane early in ciliogenesis. After basal body docking, these distal appendages constitute the transition fibers that link the basal body to the ciliary membrane/ciliary necklace (Paintrand *et al.*, 1992), that is, the fibers proposed to be constituents of a ciliary pore complex (Rosenbaum and Witman, 2002).

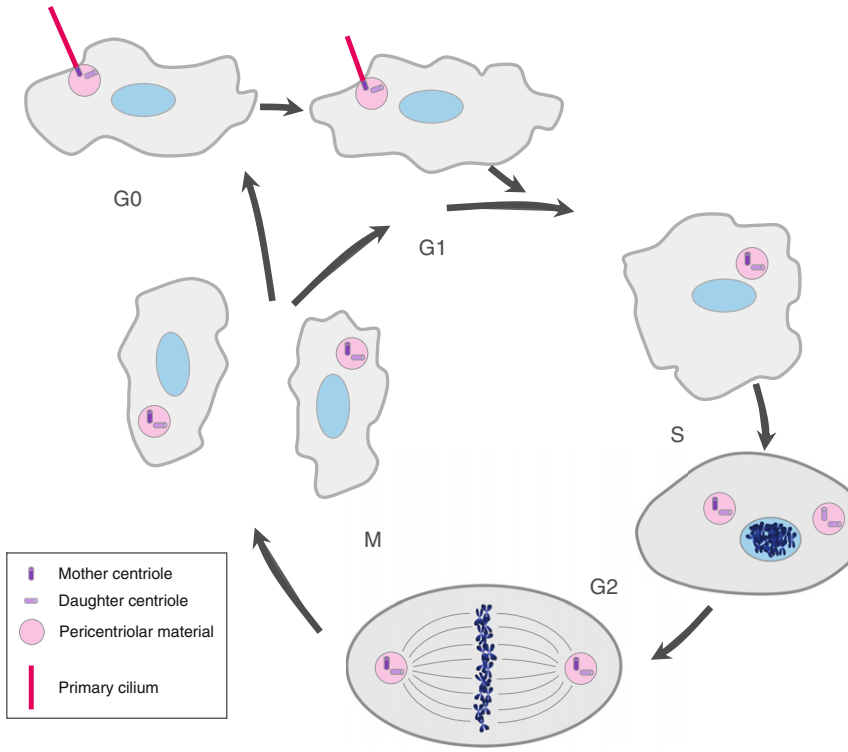

Figure 2.2 Assembly and disassembly of primary cilia are coordinated with the cell cycle. The primary cilium is formed in G1/G0 following docking of the mother centriole at the ciliary assembly site at the apical plasma membrane. Cilium elongation is mediated by IFT-dependent addition of ciliary precursors to the distal end of the mother centriole, which at this point is termed a basal body. Disassembly of the cilium prior to mitosis allows both centriole pairs to function in mitotic spindle formation.

3. General Description of Intraflagellar Transport

Since cilia and flagella lack the machinery for *de novo* protein synthesis, and since the axoneme assembles from and continuously turns over at its distal tip (Johnson and Rosenbaum, 1992; Marshall and Rosenbaum, 2001), compartmentalized ciliary assembly and maintenance relies on the constant delivery of axonemal precursors from their site of synthesis in the cell body to the axonemal assembly site at the ciliary tip, a process that is mediated by IFT (Rosenbaum and Witman, 2002).

IFT is a bidirectional MT-based motility process during which groups of large protein complexes (IFT particles) are transported along the axonemal outer doublet MTs from the base of the cilium to its distal tip by

kinesin-2 motors and then from the tip back to the cell body by cytoplasmic dynein 2 (Kozminski *et al.*, 1993, 1995; Pazour *et al.*, 1999; Porter *et al.*, 1999). During anterograde (base to tip) IFT, axonemal precursor proteins associate with the IFT machinery, which ferries the precursors to the axonemal assembly site at the ciliary tip where precursors are unloaded for assembly. Following unloading of axonemal building blocks at the ciliary tip, the ''empty'' IFT particles bind to axonemal turn over products, which are then transported back to the cell body (retrogradely) for recycling (Qin *et al.*, 2004; Fig. 2.3).

Much of our current knowledge about IFT stems from pioneering work in the biflagellate green alga *Chlamydomonas* (Cole *et al.*, 1998; Kozminski *et al.*, 1993, 1995), which remains an excellent model organism in which to study IFT, primarily because the flagella can be easily isolated and purified (Witman *et al.*, 1972), the *Chlamydomonas* genome sequence (Merchant *et al.*, 2007), flagellar proteome (Pazour *et al.*, 2005), and flagellar transcriptome (Stolc *et al.*, 2005) are known, a large number of IFT mutants are available (Cole, 2003), and IFT can be visualized *in vivo* without the aid of fluorescently tagged proteins (Kozminski *et al.*, 1993). Nevertheless, since the discovery of IFT in *Chlamydomonas* in 1993 (Kozminski *et al.*, 1993), studies in other ciliated model organisms such as *C. elegans*, *Tetrahymena*, trypanosomes, zebrafish, and the mouse have contributed significantly to our understanding of the molecular mechanisms and functions of IFT in various cellular and developmental contexts. These studies have shown that IFT is a highly conserved process required for the assembly and maintenance of almost all eukaryotic cilia and flagella, and that mutations leading to defective IFT can result in the development of severe diseases (ciliopathies) and developmental defects (Badano *et al.*, 2006; Fliegauf *et al.*, 2007; Pan *et al.*, 2005; Pazour and Rosenbaum, 2002; Sloboda, 2002; see also Section 1). Since IFT is required for assembly of the cilium proper (Rosenbaum and Witman, 2002), which in turn functions as a repository for receptors and other signaling proteins involved in controlling important cellular and developmental processes (Christensen *et al.*, 2007; Eggenschwiler and Anderson, 2007), some of the malignancies arising from defective IFT may result from the mere lack of an appropriate structure, the cilium, to harbor and coordinate specific signaling pathways. However, there is growing evidence indicating that IFT also participates *directly* in cilia-mediated signaling processes, for example, Shh signaling during vertebrate development (Eggenschwiler and Anderson, 2007) and cGMP-dependent signaling during mating in *Chlamydomonas* (Wang *et al.*, 2006), a topic that will be discussed in Section 12.

4. Discovery and Evolution of IFT

IFT was first observed by enhanced digital interference contrast (DIC) microscopy of immobilized *Chlamydomonas* flagella as a continuous, nonsaltatory movement of granular particles (IFT particles), which were seen

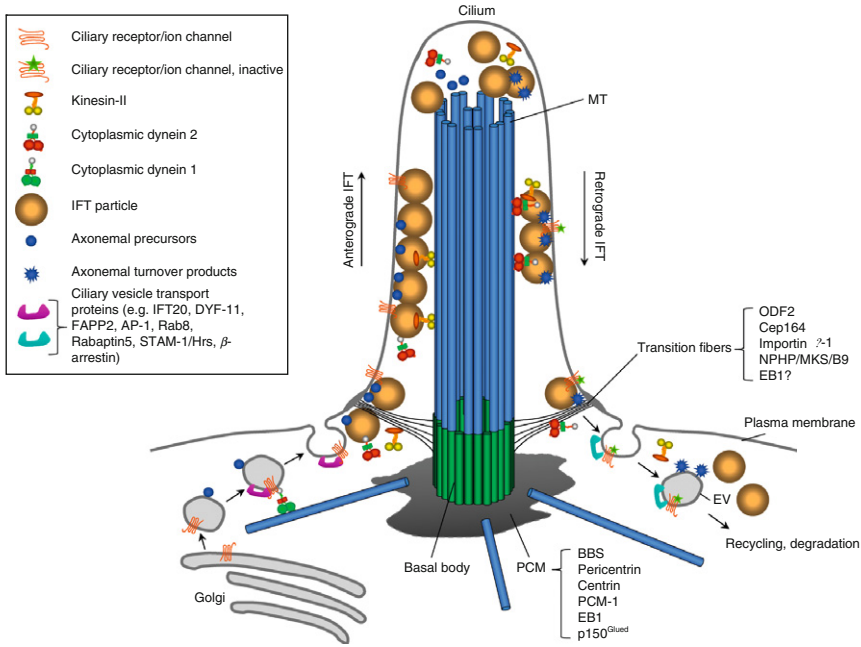

Figure 2.3 IFT and targeting of proteins to the ciliary compartment. Proteins destined for the ciliary compartment (membrane proteins as well as axonemal components) are transported in Golgi-derived vesicles to the base of the cilium where the vesicles are exocytosed and the ciliary proteins associate with IFT particles. This Golgi-to-cilium-mediated vesicle transport, which is proposed to involve cytoplasmic dynein 1 MT-based movement, depends on the IFT complex B proteins IFT20 and DYF-11, the small G protein Rab8 and associated GEFs (e.g., Rabin 8, Rabaptin 5), FAPP2, and adapter proteins such as AP-1. BBS proteins and other proteins localized in the pericentrosomal region (e.g., PCM-1, EB1, $p150^{Glued}$) may provide a link between the Golgi-derived vesicles and the transition fibers at the ciliary base and may also serve to anchor MTs at the basal body. At the ciliary base, only proteins (or protein complexes) containing specific ciliary targeting motifs are allowed access through the zone defined by the transition fibers. Selective entry of proteins into the ciliary compartment probably involves specific G proteins and GEFs that are associated with NPHPs, MKS, and B9 domain-containing proteins. Following entry into the ciliary compartment, these proteins, along with inactive cytoplasmic dynein 2, are transported anterogradely along the axoneme by kinesin-II-mediated IFT. At the ciliary tip IFT particles are remodeled, kinesin-II inactivated, and cytoplasmic dynein 2 activated. Ciliary turnover products (e.g., inactive receptors) are, in turn, transported retrogradely along ciliary axonemes by cytoplasmic dynein 2 for recycling or degradation in the cytoplasm. Recycling or turnover of ciliary membrane receptors may involve ubiquitination (e.g., via BBS proteins) and/or dephosphorylation of the receptors as well as binding to endosomal vesicle adapter proteins such as STAM-1/Hrs (Bae and Barr, 2008) or β-arrestin (Kovacs *et al.*, 2008). Figure based on references (Azimzadeh and Bornens, 2007; Leroux, 2007; Rosenbaum and Witman, 2002) as well as references cited in the text. *Abbreviations*: EV, endocytic vesicle; MT, microtubule; PCM, pericentriolar material. Figure generated by Jacob M. Schrøder, University of Copenhagen.

to move bidirectionally underneath the flagellar membrane at *ca.* 2 μm/s in the anterograde direction and at *ca.* 3.5 μm/s in the retrograde direction (Kozminski *et al.*, 1993). Studies using GFP-tagged IFT particle polypeptides or motor subunits have demonstrated similar, but sometimes faster, movement of IFT particles in cilia from a variety of other cell types and organisms, including neuronal sensory cilia in *C. elegans* (Orozco *et al.*, 1999), primary cilia of cultured IMCD and LLC-PK1 kidney cells (Follit *et al.*, 2006; Tran *et al.*, 2008), and motile flagella of *Trypanosoma brucei* (Absalon *et al.*, 2008), indicating that IFT is conserved among ciliated organisms.

By using correlative light microscopy and transmission electron microscopy (TEM) of *Chlamydomonas* flagella, Kozminski *et al.* (1995) demonstrated that the IFT particles seen to move in the DIC consist of linear arrays of lollipop-shaped structures that are connected to both the axonemal outer doublet B MTs and the flagellar membrane (Fig. 2.4). Furthermore, immunogold EM using antibodies directed against IFT particle polypeptides and motor subunits provided conclusive evidence that these lollipop-shaped structures indeed are IFT particles (Pedersen *et al.*, 2006; Sloboda and Howard, 2007).

Consistent with a conserved role for IFT in ciliary assembly and maintenance (Cole *et al.*, 1998; Perkins *et al.*, 1986), comparative genomics studies have shown that the genes encoding IFT particle polypeptides or motor subunits are highly conserved among ciliated eukaryotes, but absent from nonciliated organisms such as higher plants and fungi (Avidor-Reiss *et al.*, 2004; Li *et al.*, 2004). In addition, bioinformatic analysis of IFT particle polypeptide sequences indicated that these polypeptides display similarities to components of coat protein I (COPI) and clathrin-coated vesicles, leading to the hypothesis that IFT evolved as a specialized form of coated vesicle transport from a protocoatomer complex (Jekely and Arendt, 2006).

Most of the genes coding for IFT particle polypeptides or motor subunits have now been cloned and characterized in several different organisms and, as will be discussed below, analyses of individual IFT components have provided significant insight into the molecular mechanisms and functions of IFT, although important questions still remain to be answered. For example, how individual IFT components interact and are regulated in different organisms and under diverse physiological conditions is only now beginning to be elucidated.

5. The Canonical Anterograde IFT Motor

The canonical motor for anterograde IFT is a heterotrimeric complex belonging to the kinesin-2 family. This kinesin-2 complex, which hereafter will be referred to as kinesin-II, was first purified from sea urchin eggs and

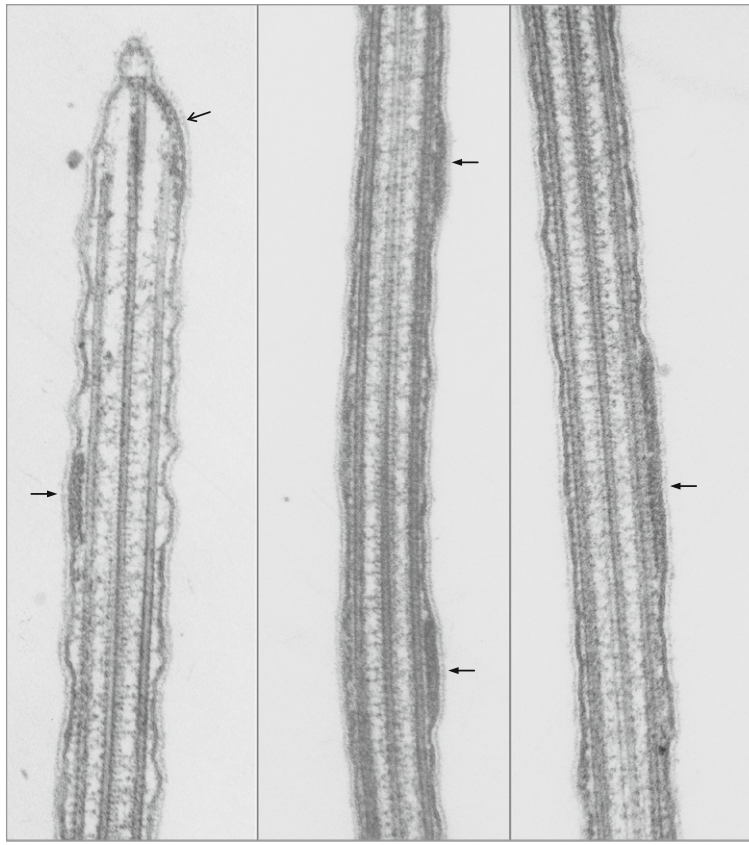

Figure 2.4 Images of IFT particles. The figure shows TEMs of longitudinal sections of *Chlamydomonas* flagella. Groups of IFT particles of variable size (closed arrows) are observed between the outer doublet B MTs and the flagellar membrane. Open arrow shows electron-dense IFT material near the flagellar tip. Courtesy of Stefan Geimer, University of Bayreuth, Germany.

consists of two motor subunits of 90 and 85 kDa, respectively, and a 100 kDa nonmotor subunit (Cole *et al.*, 1992, 1993; Scholey, 2003). In vertebrates, the 90 and 85 kDa motor subunits are termed KIF3A and KIF3B, respectively, whereas the 100 kDa nonmotor subunit is called kinesin-associated protein (KAP; Scholey, 2003). The *Chlamydomonas* orthologs of these subunits are termed FLA10 (KIF3A ortholog), FLA8 (KIF3B ortholog), and FLA3 (KAP ortholog), respectively (Table 2.1; Kozminski *et al.*, 1995; Miller *et al.*, 2005; Mueller *et al.*, 2005; Walther *et al.*, 1994). Analysis of a *Chlamydomonas* mutant, *fla10*^{ts}, which harbors a temperature-sensitive mutation in the *FLA10* gene (Adams *et al.*, 1982; Huang *et al.*, 1977; Walther *et al.*, 1994), first indicated that kinesin-II is

Table 2.1 Components of the IFT machinery

IFT polypeptide	Chlamydomonas	C. elegans	H. sapiens
Kinesin-2			
Heterotrimeric	FLA10	KRP85/ KLP-20	KIF3A
	FLA8	KRP95/ KLP-11	KIF3B
	FLA3	KAP/KAP-1	KAP
Homodimeric	?	OSM-3	KIF17
Cytoplasmic dynein 2			
	DHC1b	CHE-3	DYNC2H1/ DHC2
	D1bLIC	D2LIC/ XBX-1	DYNC2LI1/ D2LIC
	FAP133	?	WD34
	LC8/FLA14	?	?
Complex A			
IFT144	FAP66	DYF-2	WDR19
IFT140	IFT140	CHE-11	IFT140
IFT139	FAP60	ZK328.7	THM-1
IFT122A	XP_001700201.1	DAF-10	IFT122/ WDR10
IFT122B	IFT122B	IFTA-1	WDR35
IFT43	FAP118	?	?
Complex B			
IFT172	IFT172	OSM-1	IFT172
IFT88	IFT88	OSM-5	IFT88/Polaris
IFT81	IFT81	F32A6.2	IFT81
IFT80	IFT80	CHE-2	IFT80
IFT74/72	IFT74/72	C18H9.8	IFT74/72
IFT57	IFT57	CHE-13	IFT57/Hippi
IFT52	IFT52	OSM-6	IFT52/NGD5
IFT46	IFT46	DYF-6	C11orf60
IFT27	IFT27	?	RABL4
IFT20	IFT20	Y110A7A.20	IFT20
New putative IFT proteins			
	FAP259	DYF-1	TPR30A
	FAP22	DYF-3	CLUAP1
	XM_001698717.1	DYF-13	TTC26
	FAP116	DYF-11	MIP-T3

Data were obtained from GenBank and from the following references: Absalon *et al.* (2008), Blacque *et al.* (2006, 2008), Cole (2003), Li *et al.* (2008), Omori *et al.* (2008), Pazour *et al.* (2005), Pfister *et al.* (2005), Rompolas *et al.* (2007), Scholey (2003), Tran *et al.* (2008), and Tsao and Gorovsky (2008b).

required for anterograde IFT, because when mutant cells were placed at the restrictive temperature of 32 °C, IFT ceased and the flagella began to shorten. Furthermore, when the *fla10*^{ts} cells were placed at 32 °C and deflagellated, new flagella failed to form, indicating that kinesin-II and IFT are required for both assembly and maintenance of the flagella (Kozminski *et al.*, 1995). Subsequent studies in sea urchin, mouse, *Tetrahymena* and *Drosophila* showed that inactivation of kinesin-II similarly abolished ciliary assembly in these organisms (Brown *et al.*, 1999; Marszalek *et al.*, 1999; Morris and Scholey, 1997; Nonaka *et al.*, 1998; Sarpal *et al.*, 2003; Takeda *et al.*, 1999), whereas inactivation of kinesin-II in *C. elegans* prevented global ciliary assembly only when the homodimeric kinesin-2 motor OSM-3 was simultaneously inhibited, owing to partial redundancy between OSM-3 and kinesin-II in building specific subsets of sensory cilia of this organism (Scholey, 2008; Snow *et al.*, 2004). However, careful observations and measurements of motility rates of *C. elegans* kinesin-II subunits and IFT particle polypeptides, both *in vivo* and *in vitro*, demonstrated that kinesin-II indeed powers anterograde IFT in this organism (Orozco *et al.*, 1999; Ou *et al.*, 2005a; Pan *et al.*, 2006; Snow *et al.*, 2004). Such studies, along with genetic and biochemical studies in vertebrates, have also begun to shed light on the mechanisms by which kinesin-II is regulated (Burghoorn *et al.*, 2007; Pan *et al.*, 2006; Pedersen *et al.*, 2008; Scholey, 2008), a topic that will be dealt with in more detail in Section 10.

In summary, the available results suggest that heterotrimeric kinesin-II functions as the core anterograde IFT motor in virtually all ciliary systems studied to date. However, as will be discussed below, additional kinesins such as homodimeric kinesin-2 as well as other kinesin family members may cooperate with kinesin-II in the assembly of specific subsets of cilia to generate ciliary structural and functional diversity.

6. Additional Kinesin Motors Involved in Ciliogenesis

In *C. elegans* homodimeric kinesin-2 appears to cooperate with kinesin-II during anterograde IFT to mediate assembly of specific subsets of sensory cilia (Scholey, 2008; Snow *et al.*, 2004), and there is growing evidence suggesting that the deployment of "accessory" kinesins during ciliogenesis is not limited to *C. elegans* and homodimeric kinesin-2. In fact, numerous kinesins have been detected in cilia from diverse species, and some of these have been implicated in cilia assembly and maintenance, either by functioning cooperatively with kinesin-II during anterograde IFT to promote axonemal assembly, by affecting axonemal turnover and disassembly, or by transporting specific receptors and ion channels to the

ciliary membrane (Scholey, 2008). Here, we will provide a brief description of "accessory" kinesins with putative roles in IFT and/or transport of ciliary membrane proteins, namely homodimeric kinesin-2, kinesin-3, and kinesin-16 family members. For detailed descriptions of homodimeric kinesin-2 and other ciliary kinesins, see Blacque *et al.* (2008) and Scholey (2008).

A role for homodimeric kinesin-2 in IFT and cilia biogenesis was first discovered in *C. elegans* (Shakir *et al.*, 1993; Signor *et al.*, 1999b), where genetic and motility studies later revealed that the homodimeric kinesin-2 motor OSM-3 functions redundantly with heterotrimeric kinesin-2 to build the middle axonemal segment of amphid channel cilia, which consists of doublet MTs, whereas OSM-3 alone mediates assembly of the singlet MTs that constitute the distal segment of the axoneme (Snow *et al.*, 2004). In frogs, the olfactory cilia similarly contain a long segment of singlet MTs at their distal end (Reese, 1965), and biophysical measurements have indicated that cyclic nucleotide-gated channels (CNGs) are highly enriched at this site (Flannery *et al.*, 2006). It is likely that this localization of CNGs is related to homodimeric kinesin-2-mediated assembly of distal singlet axonemal MTs, because in rodents the OSM-3 homolog KIF17 has been implicated in the transport of CNGs to the olfactory cilia (Jenkins *et al.*, 2006). Furthermore, a recent study showed that morpholino-mediated knockdown of KIF17 in zebrafish inhibited the formation of photoreceptor outer segments, which essentially are modified primary cilia, and which also contain extended distal singlet axonemal MTs like *C. elegans* amphid channel and frog olfactory cilia. Importantly, the assembly of sensory cilia in the zebrafish pronephros was unaffected by KIF17 knockdown, suggesting that KIF17 plays a specific role in the assembly of cilia with extended distal singlet MTs (Insinna *et al.*, 2008).

Members of the kinesin-3 and kinesin-16 families may also be involved in ciliary transport of specific membrane proteins. In *C. elegans*, the kinesin-3 family member KLP-6 is required for ciliary localization of the polycystin-2 calcium channel that controls male mating behavior in this organism (Peden and Barr, 2005). Furthermore, an extensive phylogenetic analysis of kinesins indicated that kinesin-16 family members, as well as other kinesin subfamilies such as kinesin-17, are specific to ciliated organisms (Wickstead and Gull, 2006). Humans contain six putative kinesin-3 family members and one kinesin-16 member (KIF12; Wickstead and Gull, 2006), and preliminary results indicate that at least one of the kinesin-3 proteins, KIF13A, as well as KIF12 localize to primary cilia in diverse cultured human cells and native human embryonic tissue (S. K. Nielsen, R. I. Thorsteinsson, S. T. Christensen, and L. B. Pedersen, unpublished data). While the functional implications of these observations are still to be determined, it is of interest that KIF12 was shown to be a polycystic kidney disease modifier in the cpk mouse hinting at a possible ciliary function for this protein (Mrug *et al.*, 2005). Moreover, murine KIF13A associates directly with the AP-1 adaptor complex during transport of mannose-6-phosphate receptor-containing vesicles from the Golgi to the

plasma membrane in various cultured cell types (Nakagawa *et al.*, 2000), and in *C. elegans* AP-1 has been implicated in ciliary transport of calcium channels and odorant receptors (Bae *et al.*, 2006; Dwyer *et al.*, 2001). Therefore we hypothesize that KIF13A and KIF12, possibly in conjunction with AP-1, are involved in targeting specific receptors and/or ion channels to the ciliary membrane in vertebrate cells. Whether such targeting occurs independently or in cooperation with kinesin-II, as observed for OSM-3 in *C. elegans* (Snow *et al.*, 2004), will be interesting to investigate.

7. The Retrograde IFT Motor

The motor that powers retrograde IFT is an isoform of cytoplasmic dynein called cytoplasmic dynein 2 (previously known as cytoplasmic dynein 1b; Pfister *et al.*, 2005). This motor complex consists of at least four different subunits: a heavy chain termed DYNC2H1 that belongs to the AAA+ family of ATPases, a light intermediate chain (DYNC2LI1), a light chain (LC8), and a recently identified putative intermediate chain (IC)/WD repeat protein, which may be specific for motile cilia (Table 2.1; Pfister *et al.*, 2005; Rompolas *et al.*, 2007). The cytoplasmic dynein 2 motor subunit, DYNC2H1, was originally identified as a dynein heavy chain whose synthesis is induced by deciliation in sea urchin embryos (Gibbons *et al.*, 1994), and subsequent genetic and motility studies, primarily in *Chlamydomonas* and *C. elegans*, demonstrated that DYNC2H1 is essential for retrograde IFT. Thus null mutations in the gene encoding DYNC2H1 in these organisms led to cessation of retrograde IFT and formation of stunted cilia containing large accumulations of IFT particles, implying that transport of IFT particles out of the cilia was impaired (Pazour *et al.*, 1999; Porter *et al.*, 1999; Signor *et al.*, 1999a). In mammals, DYNC2H1 was found to localize to cilia in the brain and photoreceptors (Mikami *et al.*, 2002), and mutational inactivation of the corresponding gene led to the formation of stunted, bulbous cilia in the neuroectoderm, limb mesenchyme, and ventral node of developing mouse embryos (Huangfu and Anderson, 2005; May *et al.*, 2005). These mutant cilia are thus similar in appearance to the stunted cilia filled with IFT particles that were observed in *Chlamydomonas* mutants lacking DYNC2H1 (Pazour *et al.*, 1999; Porter *et al.*, 1999), indicating that the role of DYNC2H1 in retrograde IFT is conserved in mammals.

The DYNC2LI1 subunit of the cytoplasmic dynein 2 complex has been studied in several organisms, including *Chlamydomonas*, *C. elegans*, and mouse, and as for DYNC2H1, genetic and motility studies clearly demonstrated a requirement for this protein in retrograde IFT (Grissom *et al.*, 2002; Hou *et al.*, 2004; Perrone *et al.*, 2003; Rana *et al.*, 2004; Schafer *et al.*, 2003).

Furthermore, biochemical analyses of DYNC2LI1 from *Chlamydomonas* flagella demonstrated that DYNC2LI1 and DYNC2H1 are part of the same complex (Perrone *et al.*, 2003; Rompolas *et al.*, 2007), a complex that also contains LC8 and a recently identified WD repeat protein called FAP133 (Rompolas *et al.*, 2007). A *Chlamydomonas* mutant, *fla14*, that is defective in the gene encoding LC8 displays a phenotype consistent with defective retrograde IFT (Pazour *et al.*, 1998), and siRNA-mediated knockdown of the gene encoding the FAP133 homolog in trypanosomes resulted in flagellar dysfunction, suggesting that FAP133 may also be required for retrograde IFT (Baron *et al.*, 2007). However, the precise function of individual subunits of the cytoplasmic dynein 2 complex during retrograde IFT is still unclear. Moreover, it is possible that the complex contains additional subunits that have not yet been characterized in detail. For example, XBX-2, which contains a domain characteristic for dynein light chains of the Tctex-1 family, has been shown to undergo IFT in *C. elegans* sensory cilia (Efimenko *et al.*, 2005), but its ciliary function and relationship with the cytoplasmic dynein 2 complex are not known. However, given the large number of light chains that are involved in assembly and regulation of other dynein motor complexes, for example, outer arm dynein in *Chlamydomonas* flagella (King, 2003), it is plausible that cytoplasmic dynein 2 similarly contains multiple light chains involved in the assembly and function of the motor complex.

8. IFT Particle Polypeptides

By taking advantage of the *fla10*^{ts} mutant that harbors a temperature-sensitive mutation in the gene encoding the FLA10 motor subunit of kinesin-II (Adams *et al.*, 1982; Huang *et al.*, 1977; Walther *et al.*, 1994), IFT particle polypeptides were originally isolated from *Chlamydomonas* flagella using sucrose density gradient centrifugation and were found to consist of two separate complexes, termed A and B, containing six and ten subunits, respectively (Table 2.1; Cole *et al.*, 1998; Piperno and Mead, 1997). Genetic, motility, and bioinformatic studies in *C. elegans* subsequently led to the identification of three additional putative IFT particle polypeptides (DYF-1, DYF-3, DYF-13; Blacque *et al.*, 2005; Murayama *et al.*, 2005; Ou *et al.*, 2005a,b), and RNAi-based approaches in trypanosomes recently confirmed a role for these proteins in flagellar biogenesis (Absalon *et al.*, 2008). Homologues of DYF-1, DYF-3, and DYF-13 are also present in *Chlamydomonas* (Absalon *et al.*, 2008; Blacque *et al.*, 2008; Pazour *et al.*, 2005), but were not identified during initial purification of IFT particle polypeptides from *Chlamydomonas* flagella (Cole *et al.*, 1998; Piperno and Mead, 1997). However, genetic, motility, and RNAi-based

studies in *C. elegans* and trypanosomes suggest that these three proteins are part of complex B (Absalon *et al.*, 2008; Blacque *et al.*, 2005; Murayama *et al.*, 2005; Ou *et al.*, 2005a,b), indicating that they may dissociate from the complex during sucrose density gradient centrifugation of *Chlamydomonas* flagella extracts, as has been observed for the complex B polypeptide IFT172 (Pedersen *et al.*, 2005). Yet another putative complex B polypeptide, DYF-11, was recently identified by two separate groups (Li *et al.*, 2008; Omori *et al.*, 2008), bringing the total number of likely complex B polypeptides to 14 (Table 2.1).

The genes for all known IFT particle polypeptides have now been cloned and sequenced in multiple organisms, including *Chlamydomonas*, *C. elegans*, *Tetrahymena*, *Drosophila*, trypanosomes, zebrafish, mouse, and human (Table 2.1; Absalon *et al.*, 2008; Blacque *et al.*, 2008; Cole, 2003; Li *et al.*, 2008; Omori *et al.*, 2008; Pedersen *et al.*, 2008; Tran *et al.*, 2008; Tsao and Gorovsky, 2008b). The corresponding amino acid sequences of IFT particle polypeptides are characterized by an abundance of motifs known to be involved in protein–protein interaction, including WD repeats, TPR repeats, and coiled coils (Cole, 2003). As mentioned previously, these features may reflect an evolutionary relationship of IFT particle polypeptides with components of COPI and clathrin-coated vesicles (Jekely and Arendt, 2006), and are also consistent with a role for IFT particle proteins in transport of ciliary precursors and turn over products (Qin *et al.*, 2004). The challenge now is to determine the function of individual IFT particle polypeptides, how they interact with each other as well as with motors and cargo proteins, and how these interactions are regulated. As will be described below, significant strides have been made in terms of elucidating the functions of individual IFT polypeptides, but much still remains to be learned about their interactions and regulation.

9. Functional Analysis of IFT Particle Polypeptides: Distinct Roles of IFT Complexes A and B

The first direct evidence demonstrating that IFT particle proteins are required for cilia assembly was provided by a combination of biochemical analyses of *Chlamydomonas* flagella and genetic studies in *C. elegans*. Thus, when IFT particle polypeptides were first isolated and sequenced from *Chlamydomonas* flagella, it was discovered that some of the IFT particle polypeptides are homologous to proteins encoded by genes that when mutated inhibit assembly of sensory cilia in *C. elegans* (Cole *et al.*, 1998; Perkins *et al.*, 1986). Using similar approaches, as well as motility- and RNAi-based methods, the function of almost all known IFT particle

polypeptides has now been studied in multiple organisms. These studies have shown that in general, complete loss of any complex B polypeptide almost invariably leads to inhibition of cilia assembly, leading to the notion that complex B is required for anterograde transport of axonemal precursors into the ciliary compartment (Absalon *et al.*, 2008; Beales *et al.*, 2007; Brazelton *et al.*, 2001; Brown *et al.*, 2003; Cole, 2003; Deane *et al.*, 2001; Follit *et al.*, 2006; Fujiwara *et al.*, 1999; Haycraft *et al.*, 2001, 2003; Houde *et al.*, 2006; Huangfu *et al.*, 2003; Kobayashi *et al.*, 2007; Li *et al.*, 2008; Omori *et al.*, 2008; Pathak *et al.*, 2007; Pazour *et al.*, 2000; Pedersen *et al.*, 2005; Perkins *et al.*, 1986; Qin *et al.*, 2001, 2007; Sun *et al.*, 2004; Tran *et al.*, 2008; Tsao and Gorovsky, 2008a; Tsujikawa and Malicki, 2004). This notion is further supported by recent data from *Chlamydomonas* indicating that the complex B protein IFT46 is specifically involved in transporting outer dynein arms into flagella (Hou *et al.*, 2007). In addition to IFT46, studies in which other IFT complex B proteins have been partially depleted or inactivated have led to assignment of specific functions for IFT172, IFT27, IFT20, and DYF-11/MIP-T3. Thus IFT172 appears to be required for regulating the transition from anterograde to retrograde IFT at the flagellar tip (Pedersen *et al.*, 2005; Tsao and Gorovsky, 2008a), IFT27 is a small G protein involved in cell cycle control (Qin *et al.*, 2007), whereas IFT20 and DYF-11/MIP-T3 play a role in transport of vesicles from the Golgi to the ciliary base (Follit *et al.*, 2006; Li *et al.*, 2008; Omori *et al.*, 2008; see also Chapter 5). Furthermore, chemical crosslinking and yeast two- and three-hybrid analyses have shown that IFT81 and IFT74/72 interact directly, forming a tetrameric complex that serves as a scaffold for the intact IFT complex B (Lucker *et al.*, 2005). The remaining subunit organization within complex B as well as interactions between complex B proteins and the remaining IFT constituents are not well characterized.

In contrast to complex B polypeptides, complex A polypeptides are not always essential for building the ciliary axoneme, but rather, seem to be important for retrograde IFT. For example, the *Chlamydomonas* ts mutants *fla15*, *fla16*, and *fla17*, which contain decreased amounts of complex A polypeptides in their flagella, are able to assemble flagella but display retrograde IFT phenotypes with accumulation of IFT complex B polypeptides at the flagellar tips (Iomini *et al.*, 2001; Piperno *et al.*, 1998). In addition, mutational inactivation of IFT140/CHE-11 or IFT122A/DAF-10 in *C. elegans* results in shortened cilia that accumulate complex B polypeptides at their distal end (Collet *et al.*, 1998; Perkins *et al.*, 1986; Qin *et al.*, 2001; Schafer *et al.*, 2003). Similar phenotypes were observed when these polypeptides were inactivated in *Tetrahymena* or trypanosomes (Absalon *et al.*, 2008; Tsao and Gorovsky, 2008b), or when IFT139/THM-1 was inactivated in the mouse (Tran *et al.*, 2008). In zebrafish auditory and olfactory organs, however, inactivation of IFT140 was reported to have little observable effect on cilia structure (Tsujikawa and Malicki, 2004). While the latter

result might indicate that IFT140 is dispensable for IFT, it is also possible that there is some degree of functional overlap/redundancy among different IFT polypeptides, such that if IFT140 is not essential for stability of complex A, the remaining polypeptides may be able to compensate for its loss to some extent.

In summary, the available evidence strongly suggests that complex B is required for anterograde IFT while complex A is required for retrograde IFT. There is some evidence in the literature suggesting that complex A may also play a role in anterograde IFT, but whether this is limited to a few cellular systems is presently unclear. In *C. elegans* evidence from motility studies have suggested that complex A associates with kinesin-II during anterograde IFT and that the kinesin-II–IFT A complex in turn is linked to OSM-3–IFT B complex via Bardet-Biedl syndrome (BBS) proteins (Blacque *et al.*, 2008; Ou *et al.*, 2005a; Scholey, 2008; Snow *et al.*, 2004). Furthermore, a *Chlamydomonas* IFT140 null mutant was reported to completely lack flagella, although the evidence was preliminary (Cole, 2003). Finally, gel filtration analysis of *Chlamydomonas* flagella extracts showed that complex A cofractionates with cytoplasmic dynein 2, and that this fraction also contains kinesin-II but not IFT complex B (Rompolas *et al.*, 2007). While these findings suggest that complex A may play a role in anterograde IFT in addition to functioning in retrograde IFT, as we have previously hypothesized (Pedersen *et al.*, 2006, 2008), it is also possible that IFT A complexes are simply cargoes during anterograde IFT, docked to the kinesin-II motor. More experimental data is required to distinguish between these possibilities.

10. Regulation of IFT

Immunolocalization studies in *Chlamydomonas* demonstrated that IFT particle polypeptides, motor subunits, and flagellar precursors are highly concentrated at the base of the flagella (Cole *et al.*, 1998; Pazour *et al.*, 1999; Qin *et al.*, 2004), specifically near the site where transition fibers contact the flagellar membrane (Deane *et al.*, 2001), suggesting that IFT is initiated by the gathering of IFT components and cargo proteins at this site. Active kinesin-II presumably associates with IFT complexes to which ciliary precursors and inactive cytoplasmic dynein 2 are bound, and transports these anterogradely along the ciliary axoneme toward the tip (Iomini *et al.*, 2001; Kozminski *et al.*, 1995; Pedersen *et al.*, 2006; Qin *et al.*, 2004; Walther *et al.*, 1994). At the tip, significant remodeling of the IFT complexes takes place, precursors are unloaded for assembly, kinesin-II is inactivated, IFT complexes are remodeled, and cytoplasmic dynein 2 becomes active. Following binding of active cytoplasmic dynein 2 to complex A,

complex B reassociates with A and, presumably, ciliary turn over products, whereafter cytoplasmic dynein 2 transports everything back to the cell body for recycling (Fig. 2.3; Iomini *et al.*, 2001; Pazour *et al.*, 1999; Pedersen *et al.*, 2006; Porter *et al.*, 1999; Qin *et al.*, 2004). Since both anterograde and retrograde IFT seemingly occur at a constant rate along the flagellar shaft (Kozminski *et al.*, 1993), with slight pauses at the base and tip (Iomini *et al.*, 2001), the main points of regulation of IFT are presumably at the ciliary base and tip.

Our knowledge of the mechanisms regulating IFT motor activity and loading and unloading of IFT cargo proteins at the ciliary base and tip is still very fragmentary, although some key players involved in various aspects of IFT regulation have been identified. First, regulation of ciliary length is influenced by the rates of anterograde IFT and ciliary disassembly (Marshall and Rosenbaum, 2001), and analysis of mutations or biochemical inhibitors that affect ciliary length have revealed a number of kinases as potential regulators of the kinesin-II activity. These include MAP kinases (Bengs *et al.*, 2005; Berman *et al.*, 2003; Burghoorn *et al.*, 2007), NIMA-related kinases (Shalom *et al.*, 2008; Surpili *et al.*, 2003; White and Quarmby, 2008), GSK-3β (Wilson and Lefebvre, 2004) and associated polarity proteins (Pedersen *et al.*, 2008), which may modulate kinesin-II motor activity via phosphorylation. Second, kinesin-II activity may be regulated via conformational changes in the KAP subunit since KAP is required for localization of kinesin-II at the flagellar base and for processive movement of the motor complex along flagella in *Chlamydomonas* (Mueller *et al.*, 2005; Scholey, 2008). Third, polyglutamylation of axonemal tubulin, mediated by the DYF-1 protein and an associated tubulin polyglutamylase (Ttll6), impairs the velocity of kinesin-2 motors in *C. elegans* amphid channel cilia, suggesting that tubulin polyglutamylation may regulate kinesin-2 motor processivity in the cilia (Ou *et al.*, 2005a; Pathak *et al.*, 2007; Scholey, 2008). Finally, in *C. elegans* amphid channel cilia, and potentially other types of cilia that deploy both heterotrimeric and homodimeric kinesin-2 motors during anterograde IFT, BBS proteins appear to play a critical role keeping the two kinesin-2 motors together (Blacque *et al.*, 2004; Ou *et al.*, 2005a; Pan *et al.*, 2006). Thus regulation of kinesin-2 motor activity appears to be quite complex involving a variety of different regulatory mechanisms (e.g., phosphorylation, conformational changes, tubulin modifications) and molecules, but how these different regulatory pathways and molecules interact and are coordinated during the IFT process is unclear. Further complicating the picture are findings suggesting that ciliary length may be regulated not only by changes in motor activity, but also via changes in the association between IFT particles and cargo proteins (Mukhopadhyay *et al.*, 2008; Pan and Snell, 2005; see also Section 12).

While the regulation of anterograde IFT and kinesin-II activity appears to be very complex and not yet fully understood, the regulation of

cytoplasmic dynein 2 and retrograde IFT is even more obscure. This may in part be because the motor complex has been difficult to purify biochemically (Rompolas *et al.*, 2007) and because the complex may contain additional components (e.g., LCs) that have not yet been identified (see Section 7). Nevertheless, it seems that in terms of docking of the dynein motor complex onto the anterograde IFT machinery, LC8 plays a critical role, because in a *Chlamydomonas* LC8 mutant, the dynein motor complex fails to enter the flagellar compartment (Rompolas *et al.*, 2007). Furthermore, in terms of regulating the transition between anterograde and retrograde IFT at the flagellar tip, IFT172 seems to play an essential role (Pedersen *et al.*, 2005; Tsao and Gorovsky, 2008a), possibly in conjunction with the small MT-associated protein EB1 (Pedersen *et al.*, 2005), which localizes to the flagellar tip and basal bodies in *Chlamydomonas* (Pedersen *et al.*, 2003). Interestingly, we recently found that EB1 is required for assembly of primary cilia in mouse fibroblasts, although in these cells, the effect of EB1 on ciliogenesis appeared to be mainly at the level of the basal body and not the ciliary tip (Schrøder *et al.*, 2007). Consistent with these findings, EB1 was found to localize to the connecting cilia of rat photoreceptors (Schmitt and Wolfrum, 2001), which is a structure that is similar to the transition zone of other cilia and flagella (Insinna and Besharse, 2008). The EB1-binding partner $p150^{Glued}$ interacts with BBS4 (Kim *et al.*, 2004) as well as with the centrosomal protein Cep290/NPNP6 (Chang *et al.*, 2006), suggesting that $p150^{Glued}$ and EB1 may play a role in regulating ciliogenesis at the level of the transition zone/ciliary pore complex region (see Section 11), although further experiments are required to test this hypothesis. Since mammalian cells contain three EB1-related proteins (EB1, EB2, and EB3; Lansbergen and Akhmanova, 2006) it is also possible that EB2 and/or EB3 participate in some aspects of ciliogenesis, for example, IFT regulation at the ciliary tip. Indeed, preliminary results suggest that EB3, but not EB2, is required for ciliogenesis in cultured human fibroblasts, although the mechanism involved is still unknown (J. M. Schrøder and L. B. Pedersen, unpublished data). Identification of proteins that interact with IFT172 and EB proteins may provide more clues about the mechanisms by which these proteins affect ciliogenesis at the ciliary tip and base.

11. Targeting of Proteins to the Ciliary Compartment: Clues from Ciliary Disease Genes

Although the ciliary membrane is continuous with the plasma membrane of the cell, it is clear from numerous lines of evidence that the ciliary compartment is enriched for proteins, for example, specific membrane receptors and ion channels, that are absent or present in very low amounts

in the remaining parts of the cell (Christensen *et al.*, 2007; Rosenbaum and Witman, 2002). Furthermore, specific ciliary targeting sequences/motifs have been identified in a number of ciliary membrane proteins (for reviews, see Christensen *et al.*, 2007 and Chapter 5), implying that cells possess mechanisms for selectively sorting and targeting proteins to the ciliary compartment. How is this specific sorting and targeting of ciliary proteins achieved? Based on EM observations in protists (Bouck, 1971) and cultures of vertebrate fibroblasts and smooth muscle cells (Sorokin, 1962), as well as studies in vertebrate photoreceptors (Deretic and Papermaster, 1991), it was proposed that specific ciliary membrane proteins synthesized on the rough endoplasmatic reticulum are trafficked through the Golgi apparatus and then transported in post-Golgi vesicles to the base of the cilium where the vesicles are exocytosed at the site where ciliary transition fibers contact the membrane, and ciliary proteins then become associated with the IFT machinery. Furthermore, it was proposed that axonemal proteins synthesized in the cytoplasm associate peripherically with post-Golgi vesicles and are transported to the ciliary compartment in a similar fashion (Rosenbaum and Witman, 2002). Consistent with this view, immunogold EM in *Chlamydomonas* revealed that IFT particle polypeptides are highly concentrated at the ciliary transition fiber-membrane contact site (Deane *et al.*, 2001), suggesting that this site indeed is where proteins destined for the ciliary compartment associate with the IFT machinery prior to being transported across the barrier formed by the transition fibers and into the cilium proper. Recent evidence from vertebrate systems as well as nematodes strongly support this scenario for ciliary protein targeting, and have also begun to shed light on the molecular mechanisms involved in different aspects of this process (summarized in Fig. 2.3).

In terms of delivery and fusion of Golgi-derived vesicles to the ciliary base, several key players involved have now been identified, including the IFT complex B proteins IFT20 and DYF-11/MIP-T3 (Follit *et al.*, 2006; Li *et al.*, 2008; Omori *et al.*, 2008), small GTPases such as Rab8 (Nachury *et al.*, 2007), the pleckstrin homology domain-containing protein FAPP2 (Vieira *et al.*, 2006), BBS proteins and their binding partners (Nachury *et al.*, 2007), as well as the AP-1 adaptor complex (Bae *et al.*, 2006; Dwyer *et al.*, 2001; for reviews, see Leroux, 2007; Reiter and Mostov, 2006 and Chapter 5). For example, subcellular localization and siRNA-based studies in cultured vertebrate cells indicated that IFT20 colocalizes with Golgi markers and is required for targeting of polycystin-2 to the ciliary compartment (Follit *et al.*, 2006). Furthermore, recent studies in *C. elegans* and zebrafish showed that IFT20 interacts directly with DYF-11/MIP-T3, which in turn was found to be essential for early stages of ciliogenesis, and which interacts, at least indirectly, with the endocytotic regulator Rabaptin 5 as well as with Rab8 (Kunitomo and Iino, 2008; Li *et al.*, 2008; Omori *et al.*, 2008). Rab8 localizes to cilia and has been implicated in ciliary membrane

transport in vertebrates and *C. elegans* (Moritz *et al.*, 2001; Mukhopadhyay *et al.*, 2008; Nachury *et al.*, 2007). In the latter organism, it was suggested that Rab8-mediated vesicle trafficking to the cilium might be controlled by sensory input from specific receptors and ion channels located in the ciliary membrane (Mukhopadhyay *et al.*, 2008). Rab8 also interacts with a complex of at least seven BBS proteins, termed the BBSome, which was proposed to function in ciliary membrane biogenesis via a mechanism that might also include the pericentriolar matrix protein PCM-1 (Nachury *et al.*, 2007).

BBS is a genetically heterogeneous disorder characterized by cognitive impairment, obesity, polydactyly, renal cystic disease, and retinal degeneration. So far a total of 12 BBS genes have been identified, most of which have been characterized in detail at the molecular level (for reviews, see Blacque and Leroux, 2006; Tobin and Beales, 2007). Of particular interest here is a recent study demonstrating that mice lacking the genes encoding BBS2 or BBS4 exhibit a lack of ciliary localization of somatostatin receptor type 3 (Sstr3) and melanin-concentrating hormone receptor 1 (Mchr1) in the brain, indicating that BBS proteins are essential for targeting of specific receptors to the ciliary membrane (Berbari *et al.*, 2008). Consistent with these results, the ciliary TRPV channel OSM-9 was found to be mislocalized in *C. elegans bbs* mutants (Tan *et al.*, 2007). However, BBS proteins have also been suggested to participate in the regulation of anterograde IFT in certain subsets of *C. elegans* sensory cilia via coordination of heterotrimeric and homodimeric kinesin-2 motors (Ou *et al.*, 2005a), and several BBS proteins have been implicated in ubiquitination (Chiang *et al.*, 2006) and proteasome-mediated degradation of β-catenin (Gerdes *et al.*, 2007), suggesting that BBS proteins have multiple functions related to ciliary signaling, spanning from ciliary membrane vesicle/receptor transport and coordination of kinesin-2 motors to proteasome-mediated degradation of Wnt pathway components. This is in line with the pleiotropic clinical manifestations of BBS patients (Blacque and Leroux, 2006; Tobin and Beales, 2007).

As mentioned previously, the Golgi-derived vesicles destined for the ciliary compartment are probably exocytosed at or near the site where transition fibers contact the ciliary membrane (Rosenbaum and Witman, 2002), which is the site where IFT proteins accumulate (Deane *et al.*, 2001). The transition fibers appear to be derived from the distal appendages of the mother centriole (Paintrand *et al.*, 1992), and hence the formation of these appendages is likely to be essential for ciliogenesis. Indeed, studies in mammalian cells suggested that the Odf2 and Cep164 proteins are involved in forming the distal appendages on the mother centriole and depletion of these proteins resulted in failure to assemble primary cilia (Graser *et al.*, 2007; Ishikawa *et al.*, 2005). Interestingly, Odf2 was found to interact directly with Rab8 in yeast-2-hybrid and pull-down assays, suggesting a

direct link between the Odf2-containing distal appendages and the post-Golgi vesicle docking site at the ciliary base (Yoshimura *et al.*, 2007). Additional components of these appendages/transition fibers may include importin β-1, an essential component of the nuclear pore complex that interacts with Ran, regulates nuclear import and export and is required for ciliogenesis in MDCK cells (Fan *et al.*, 2007), as well as centrosomal proteins such as pericentrin (Jurczyk *et al.*, 2004; for a review on centrosomal proteins involved in ciliogenesis, see Pedersen *et al.*, 2008). Furthermore, emerging evidence suggests that several proteins associated with nephronophthisis, MKS, and related disorders such as Joubert and Senior-Løken syndromes also play a critical role in ciliogenesis at the ciliary base (for recent reviews, see Lehman *et al.*, 2008; Salomon *et al.*, 2008; von Schnakenburg *et al.*, 2007). In mammals several of the nephrocystins (NPHPs) such as NPHP-4, -5, -6, and -8 as well as some of their binding partners localize to connecting cilia of photoreceptors (Arts *et al.*, 2007; Khanna *et al.*, 2005; Salomon *et al.*, 2008), and for some of the NPHPs a requirement in ciliogenesis has been established. For example, siRNA-mediated depletion of NPHP-6/Cep290 impairs ciliogenesis in cultured retinal pigment epithelial cells (Graser *et al.*, 2007). Further, in *C. elegans* NPHP-1 and NPHP-4 localize to basal bodies and transition zones of sensory cilia, and mutant animals defective in the genes encoding these proteins display cilia in which certain GFP-tagged IFT proteins display abnormal and reduced ciliary localization patterns, suggesting a role for NPHP-1 and NPHP-4 in regulating ciliary access of the IFT machinery (Jauregui *et al.*, 2008). How might NPHPs regulate such selective entry of proteins into the ciliary compartment? At the nuclear pore access of proteins harboring nuclear localization signals depends on a G protein that, when in its GDP state, binds the protein to be transferred and releases it again in the nucleus when a nuclear guanine nucleotide exchange factor (GEF) converts the G protein to its GTP state (Chook and Blobel, 1999). Given that GEFs and small G proteins have been localized to cilia and ciliary transition zones in a variety of cell types and organisms (Hong *et al.*, 2003; Leroux, 2007; Nair *et al.*, 1999), and that some of the mammalian NPHPs (e.g., NPHP-4, NPHP-5, and NPHP-8) interact with the putative GEF RPGR-ORF15 that is also localized near the ciliary transition zone (Arts *et al.*, 2007; Khanna *et al.*, 2005), one can speculate that NPHPs and associated GEFs may similarly regulate access of ciliary proteins at the base of the cilium via small G proteins.

While the available evidence suggests a role for NPHPs in regulating ciliary access of proteins at the ciliary base, the molecular mechanisms involved are likely to be complex and involve a number of additional proteins that are only now beginning to be uncovered. Thus a recent study in *C. elegans* (Williams *et al.*, 2008) showed that NPHP-1 and NPHP-4 function redundantly with a complex of three B9 domain-containing proteins (XBX-7, TZA-1, and TZA-2), which are homologous

to human MKS1, B9D2/Stumpy, and B9D1 proteins, respectively. Williams and colleagues found that *C. elegans* XBX-7, TZA-1, and TZA-2 localize to the base of sensory cilia, but animals harboring mutations in any one of the three genes encoding these proteins do not have any overt ciliary structural or functional defects. Although the lack of ciliary defects in the mutant animals could be explained by partial retention of B9 protein function (the mutants were not null mutants), when mutations in the genes encoding XBX-7, TZA-1, or TZA-2 were combined with mutations in *NPHP-1* or *NPHP-4*, severe ciliary structural defects were observed (Williams *et al.*, 2008). Consistent with a role for B9 domain proteins in ciliogenesis, loss of the mammalian homologs of XBX-7 (MKS1) or TZA-1 (Stumpy) impairs formation of primary cilia (Dawe *et al.*, 2007b; Town *et al.*, 2008). In addition, for MKS1 as well as for its binding partner MKS3 it was shown that depletion of the proteins impaired migration of centrioles to the ciliary assembly site at the apical plasma membrane, suggesting a role for these MKS proteins in centriole migration and/or docking (Dawe *et al.*, 2007b). Whether MKS proteins contribute to centriole migration/docking in all cell types is uncertain and may depend on cell morphology, because primary fibroblasts from patients with mutations in the newly identified *MKS6/CC2D2A* gene lack primary cilia but display normal localization of centrioles at the apical plasma membrane (Tallila *et al.*, 2008). As more and more ciliary disease genes are being identified and characterized, it is also becoming evident that the phenotype/clinical feature of a specific disease-causing mutation varies greatly depending on the nature of the mutation (Lehman *et al.*, 2008; Marshall, 2008). Consequently, determining the molecular mechanism(s) by which individual ciliary disease proteins function will require careful examination of these proteins at the genetic, biochemical, and cellular levels.

12. IFT and Cilia-Mediated Signaling

The ciliary membrane is a repository for receptors and ion channels involved in a number of signaling pathways that control cell growth, behavior, and development, including the vertebrate Shh, PDGFRα, polycystin, and Wnt pathways (Christensen *et al.*, 2007; Eggenschwiler and Anderson, 2007; Pazour and Witman, 2003; Singla and Reiter, 2006). Given the requirement for IFT in building the ciliary axoneme it is perhaps not surprising that mutations in genes encoding IFT particle or motor subunits adversely affect signaling mediated via these pathways (Corbit *et al.*, 2008; Huangfu *et al.*, 2003; Pazour *et al.*, 2000; Schneider *et al.*, 2005). Emerging evidence suggests, however, that IFT also plays a more direct role in cilia-mediated signaling. For example, during mating in

Chlamydomonas, targeting of a cGMP-dependent protein kinase to a discrete flagellar compartment was shown to depend directly on IFT (Wang *et al.*, 2006), and studies of the Shh pathway in vertebrates also strongly support a direct role for IFT in signaling (reviewed in Scholey and Anderson, 2006). One way that IFT could participate directly in signaling is by transporting specific signaling proteins (e.g., receptors and ion channels) into the ciliary compartment in response to environmental cues. As discussed above and summarized in Fig. 2.3, there is ample evidence suggesting that IFT, in conjunction with a number of vesicle transport and adapter proteins such as Rab8 and BBS proteins, is directly involved in transporting specific membrane proteins into the ciliary compartment. Other lines of evidence suggest that such IFT-mediated transport could be modulated in response to environmental cues. For example, recent work in *C. elegans* indicated that sensory signaling input from specific ciliary chemosensory signaling molecules is required to maintain the structure and architecture of AWB olfactory neuron cilia, and that this signaling-mediated remodeling of cilia architecture depends on kinesin-II as well as on BBS proteins and Rab8 (Mukhopadhyay *et al.*, 2008). Further support for the idea that IFT-mediated ciliary targeting of specific signaling molecules may be modulated by environmental signals are provided by observations that IFT mediates vectorial movement of TRPV channels within the ciliary membrane of *C. elegans* sensory neurons (Qin *et al.*, 2005) and that the 7TM receptor protein Smoothened shuttles into and out of the cilia in response to Shh ligand (Corbit *et al.*, 2005; Rohatgi *et al.*, 2007). The mechanisms by which environmental signals affect IFT-mediated transport of specific signaling molecules are still elusive, however, but appear not to involve alterations of IFT motor activity (Mukhopadhyay *et al.*, 2008). Rather, it is likely that environmental cues affect the loading/unloading of specific IFT cargoes at the ciliary base and tip. Indeed, there is evidence from *Chlamydomonas* indicating that signals that trigger disassembly of the flagellum can regulate the association of axonemal proteins with IFT particles at the flagellar base and tip (Pan and Snell, 2005). How such environmental signals are translated to regulate IFT–cargo protein interactions is, however, unclear.

While the role for IFT in targeting specific signaling proteins to the ciliary compartment appears to be important for cilia-mediated signal transduction, the downregulation, processing and/or removal of ciliary signaling proteins is likely to be equally important. Indeed, partial or transient inhibition of IFT in *Chlamydomonas*, *C. elegans*, or vertebrate cells was shown to result in accumulation of polycystin-2 in the cilium, indicating that IFT plays a role in removing polycystin-2 from the ciliary compartment (Bae and Barr, 2008; Huang *et al.*, 2007; Pazour *et al.*, 2002). Moreover, evidence from vertebrates indicated a requirement for IFT in the processing of Gli transcription factors of the Shh pathway, which directly impinges on transcription of Shh-responsive genes (Haycraft *et al.*, 2005). The notion

that IFT-mediated processing of ciliary signaling components affects transcription of downstream target genes is also supported by studies indicating that the ciliary polycystin-1 receptor becomes processed following mechanical stimulation, and subsequently translocates to the nucleus to directly regulate transcription via the transcription factor STAT6 and its coactivator P100 (Chauvet *et al.*, 2004; Low *et al.*, 2006). The extent to which IFT is involved in this processing, however, and the exact mechanisms by which such IFT-mediated processing might occur are not clear.

13. Conclusions and Perspectives

Since its discovery in 1993 (Kozminski *et al.*, 1993), significant progress has been made toward understanding the molecular mechanisms and functions of IFT. Virtually all the IFT particle polypeptides and motor subunits have been identified, and most of them characterized at the functional level in multiple organisms. However, we still know relatively little about how IFT is regulated; what is clear, however, is that IFT regulation is complex involving many different molecules and mechanisms that may affect motor activity and/or the association between IFT particles and their cargo at the ciliary base and tip. What is also quite clear is that there is an intimate relationship between IFT regulation, environmental signals, and the cell cycle, which is only now beginning to be uncovered.

One important aspect of IFT regulation involves the association of ciliary precursors and IFT particles at the ciliary base, which likely is controlled, at least in part, by sensory input via the ciliary membrane. Combined efforts from many different groups have revealed a growing list of disease-related proteins, including BBS, NPHPs, and MKS-associated proteins, which may be critical for regulating access of proteins into the ciliary compartment and potentially the association of such proteins with IFT particles. A future challenge will be to define the exact interactions between the large number of proteins that constitute or function in the vicinity of the ciliary transition fibers, and to determine how these interactions are regulated in response to environmental and cellular signals. To this end, it will be necessary to employ biochemical methods such as fractionation and sucrose density gradient analysis of isolated ciliary preparations, for which organisms like *Chlamydomonas* are ideally suited. Finally, it is becoming apparent that while IFT plays a critical role in bringing precursors and signaling proteins into the ciliary compartment, it may have an equally important role in their subsequent processing and removal. There is good evidence that processed ciliary proteins (e.g., receptors and transcription factors) can affect transcription of specific target genes. This crosstalk

between the cilium and nucleus will be an interesting avenue for future research. Among candidate proteins involved in regulating transcription in response to ciliary signals are proteins associated with the development of ciliopathies, but which do not localize to the cilium, such as the recently identified Seahorse protein from zebrafish (Kishimoto *et al.*, 2008).

ACKNOWLEDGMENTS

Research in the authors' laboratories is supported by grants from the Danish Natural Science Research Council (nr. 272-05-0411 and 272-07-0503), The Novo Nordisk Foundation, and The Lundbeck Foundation (LBP) as well as the National Institutes of Health (GMO14642; JLR). We are grateful to Drs. Karl F. Lechtreck, George B. Witman, David R. Mitchell, Cynthia G. Jensen, Randy H. Cardon, and Stefan Geimer for EM images of cilia and flagella and to Jacob M. Schrøder for Figs. 2.1 and 2.3.

REFERENCES

Absalon, S., Blisnick, T., Kohl, L., Toutirais, G., Dore, G., Julkowska, D., Tavenet, A., and Bastin, P. (2008). Intraflagellar transport and functional analysis of genes required for flagellum formation in trypanosomes. *Mol. Biol. Cell* **19,** 929–944.

Adams, G. M. W., Huang, B., and Luck, D. J. L. (1982). Temperature-sensitive, assembly-defective flagella mutants of *Chlamydomonas reinhardtii*. *Genetics* **100,** 579–586.

Arts, H. H., Doherty, D., van Beersum, S. E., Parisi, M. A., Letteboer, S. J., Gorden, N. T., Peters, T. A., Marker, T., Voesenek, K., Kartono, A., Ozyurek, H., Farin, F. M., *et al.* (2007). Mutations in the gene encoding the basal body protein RPGRIP1L, a nephrocystin-4 interactor, cause Joubert syndrome. *Nat. Genet.* **39,** 882–888.

Avidor-Reiss, T., Maer, A. M., Koundakjian, E., Polyanovsky, A., Keil, T., Subramaniam, S., and Zuker, C. S. (2004). Decoding cilia function: Defining specialized genes required for compartmentalized cilia biogenesis. *Cell* **117,** 527–539.

Azimzadeh, J., and Bornens, M. (2007). Structure and duplication of the centrosome. *J. Cell Sci.* **120,** 2139–2142.

Badano, J. L., Mitsuma, N., Beales, P. L., and Katsanis, N. (2006). The ciliopathies: An emerging class of human genetic disorders. *Annu. Rev. Genomics Hum. Genet.* **7,** 125–148.

Bae, Y. K., and Barr, M. M. (2008). Sensory roles of neuronal cilia: Cilia development, morphogenesis, and function in *C. elegans*. *Front. Biosci.* **13,** 5959–5974.

Bae, Y. K., Qin, H., Knobel, K. M., Hu, J., Rosenbaum, J. L., and Barr, M. M. (2006). General and cell-type specific mechanisms target TRPP2/PKD-2 to cilia. *Development* **133,** 3859–3870.

Baron, D. M., Ralston, K. S., Kabututu, Z. P., and Hill, K. L. (2007). Functional genomics in *Trypanosoma brucei* identifies evolutionarily conserved components of motile flagella. *J. Cell Sci.* **120,** 478–491.

Barr, M. M., and Sternberg, P. W. (1999). A polycystic kidney-disease gene homologue required for male mating behaviour in *C. elegans*. *Nature* **401,** 386–389.

Beales, P. L., Bland, E., Tobin, J. L., Bacchelli, C., Tuysuz, B., Hill, J., Rix, S., Pearson, C. G., Kai, M., Hartley, J., Johnson, C., Irving, M., *et al.* (2007). IFT80, which encodes a conserved intraflagellar transport protein, is mutated in Jeune asphyxiating thoracic dystrophy. *Nat. Genet.* **39,** 727–729.

Bengs, F., Scholz, A., Kuhn, D., and Wiese, M. (2005). LmxMPK9, a mitogen-activated protein kinase homologue affects flagellar length in *Leishmania mexicana*. *Mol. Microbiol.* **55,** 1606–1615.

Berbari, N. F., Lewis, J. S., Bishop, G. A., Askwith, C. C., and Mykytyn, K. (2008). Bardet-Biedl syndrome proteins are required for the localization of G protein-coupled receptors to primary cilia. *Proc. Natl Acad. Sci. USA* **105,** 4242–4246.

Berman, S. A., Wilson, N. F., Haas, N. A., and Lefebvre, P. A. (2003). A novel MAP kinase regulates flagellar length in *Chlamydomonas*. *Curr. Biol.* **13,** 1145–1149.

Blacque, O. E., and Leroux, M. R. (2006). Bardet-Biedl syndrome: An emerging pathomechanism of intracellular transport. *Cell. Mol. Life Sci.* **63,** 2145–2161.

Blacque, O. E., Reardon, M. J., Li, C., McCarthy, J., Mahjoub, M. R., Ansley, S. J., Badano, J. L., Mah, A. K., Beales, P. L., Davidson, W. S., Johnsen, R. C., Audeh, M., *et al.* (2004). Loss of *C. elegans* BBS-7 and BBS-8 protein function results in cilia defects and compromised intraflagellar transport. *Genes Dev.* **18,** 1630–1642.

Blacque, O. E., Perens, E. A., Boroevich, K. A., Inglis, P. N., Li, C., Warner, A., Khattra, J., Holt, R. A., Ou, G., Mah, A. K., McKay, S. J., Huang, P., *et al.* (2005). Functional genomics of the cilium, a sensory organelle. *Curr. Biol.* **15,** 935–941.

Blacque, O. E., Li, C., Inglis, P. N., Esmail, M. A., Ou, G., Mah, A. K., Baillie, D. L., Scholey, J. M., and Leroux, M. R. (2006). The WD repeat-containing protein IFTA-1 is required for retrograde intraflagellar transport. *Mol. Biol. Cell* **17,** 5053–5062.

Blacque, O. E., Cevik, S., and Kaplan, O. I. (2008). Intraflagellar transport: From molecular characterisation to mechanism. *Front. Biosci.* **13,** 2633–2652.

Bouck, G. B. (1971). The structure, origin, isolation, and composition of the tubular mastigonemes of the *Ochromonas* flagellum. *J. Cell Biol.* **50,** 362–384.

Brazelton, W. J., Amundsen, C. D., Silflow, C. D., and Lefebvre, P. A. (2001). The *bld1* mutation identifies the *Chlamydomonas osm-6* homolog as a gene required for flagellar assembly. *Curr. Biol.* **11,** 1591–1594.

Brown, J. M., Marsala, C., Kosoy, R., and Gaertig, J. (1999). Kinesin-II is preferentially targeted to assembling cilia and is required for ciliogenesis and normal cytokinesis in *Tetrahymena*. *Mol. Biol. Cell* **10,** 3081–3096.

Brown, J. M., Fine, N. A., Pandiyan, G., Thazhath, R., and Gaertig, J. (2003). Hypoxia regulates assembly of cilia in suppressors of *Tetrahymena* lacking an intraflagellar transport subunit gene. *Mol. Biol. Cell* **14,** 3192–3207.

Burghoorn, J., Dekkers, M. P., Rademakers, S., de Jong, T., Willemsen, R., and Jansen, G. (2007). Mutation of the MAP kinase DYF-5 affects docking and undocking of kinesin-2 motors and reduces their speed in the cilia of *Caenorhabditis elegans*. *Proc. Natl Acad. Sci. USA* **104,** 7157–7162.

Chang, B., Khanna, H., Hawes, N., Jimeno, D., He, S., Lillo, C., Parapuram, S. K., Cheng, H., Scott, A., Hurd, R. E., Sayer, J. A., Otto, E. A., *et al.* (2006). In-frame deletion in a novel centrosomal/ciliary protein CEP290/NPHP6 perturbs its interaction with RPGR and results in early-onset retinal degeneration in the rd16 mouse. *Hum. Mol. Genet.* **15,** 1847–1857.

Chauvet, V., Tian, X., Husson, H., Grimm, D. H., Wang, T., Hiesberger, T., Igarashi, P., Bennett, A. M., Ibraghimov-Beskrovnaya, O., Somlo, S., and Caplan, M. J. (2004). Mechanical stimuli induce cleavage and nuclear translocation of the polycystin-1 C terminus. *J. Clin. Invest.* **114,** 1433–1443.

Chiang, A. P., Beck, J. S., Yen, H. J., Tayeh, M. K., Scheetz, T. E., Swiderski, R. E., Nishimura, D. Y., Braun, T. A., Kim, K. Y., Huang, J., Elbedour, K., Carmi, R., *et al.* (2006). Homozygosity mapping with SNP arrays identifies TRIM32, an E3 ubiquitin ligase, as a Bardet-Biedl syndrome gene (BBS11). *Proc. Natl Acad. Sci. USA* **103,** 6287–6292.

Chook, Y. M., and Blobel, G. (1999). Structure of the nuclear transport complex karyopherin-beta2-Ran × GppNHp. *Nature* **399,** 230–237.

Christensen, S. T., Pedersen, L. B., Schneider, L., and Satir, P. (2007). Sensory cilia and integration of signal transduction in human health and disease. *Traffic* **8,** 97–109.

Cole, D. G. (2003). The intraflagellar transport machinery of *Chlamydomonas reinhardtii*. *Traffic* **4,** 1–8.

Cole, D. G., Cande, W. Z., Baskin, R. J., Skoufias, D. A., Hogan, C. J., and Scholey, J. M. (1992). Isolation of a sea urchin egg kinesin-related protein using peptide antibodies. *J. Cell Sci.* **101,** 291–301.

Cole, D. G., Chinn, S. W., Wedaman, K. P., Hall, K., Vuong, T., and Scholey, J. M. (1993). Novel heterotrimeric kinesin-related protein purified from sea urchin eggs. *Nature* **366,** 268–270.

Cole, D. G., Diener, D. R., Himelblau, A. L., Beech, P. L., Fuster, J. C., and Rosenbaum, J. L. (1998). *Chlamydomonas* kinesin-II-dependent intraflagellar transport (IFT): IFT particles contain proteins required for ciliary assembly in *Caenorhabditis elegans* sensory neurons. *J. Cell Biol.* **141,** 993–1008.

Collet, J., Spike, C. A., Lundquist, E. A., Shaw, J. E., and Herman, R. K. (1998). Analysis of *osm-6*, a gene that affects sensory cilium structure and sensory neuron function in *C. elegans*. *Genetics* **148,** 187–200.

Corbit, K. C., Aanstad, P., Singla, V., Norman, A. R., Stainier, D. Y., and Reiter, J. F. (2005). Vertebrate Smoothened functions at the primary cilium. *Nature* **437,** 1018–1021.

Corbit, K. C., Shyer, A. E., Dowdle, W. E., Gaulden, J., Singla, V., Chen, M. H., Chuang, P. T., and Reiter, J. F. (2008). Kif3a constrains beta-catenin-dependent Wnt signalling through dual ciliary and non-ciliary mechanisms. *Nat. Cell Biol.* **10,** 70–76.

Dabdoub, A., and Kelley, M. W. (2005). Planar cell polarity and a potential role for a Wnt morphogen gradient in stereociliary bundle orientation in the mammalian inner ear. *J. Neurobiol.* **64,** 446–457.

Davenport, J. R., and Yoder, B. K. (2005). An incredible decade for the primary cilium: A look at a once-forgotten organelle. *Am. J. Physiol. Renal Physiol.* **289,** F1159–F1169.

Dawe, H. R., Farr, H., and Gull, K. (2007a). Centriole/basal body morphogenesis and migration during ciliogenesis in animal cells. *J. Cell Sci.* **120,** 7–15.

Dawe, H. R., Smith, U. M., Cullinane, A. R., Gerrelli, D., Cox, P., Badano, J. L., Blair-Reid, S., Sriram, N., Katsanis, N., Attie-Bitach, T., Afford, S. C., Copp, A. J., *et al.* (2007b). The Meckel-Gruber syndrome proteins MKS1 and meckelin interact and are required for primary cilium formation. *Hum. Mol. Genet.* **16,** 173–186.

Deane, J. A., Cole, D. G., Seeley, E. S., Diener, D. R., and Rosenbaum, J. L. (2001). Localization of intraflagellar transport protein IFT52 identifies basal body transitional fibers as the docking site for IFT particles. *Curr. Biol.* **11,** 1586–1590.

Deretic, D., and Papermaster, D. S. (1991). Polarized sorting of rhodopsin on post-Golgi membranes in frog retinal photoreceptor cells. *J. Cell Biol.* **113,** 1281–1293.

Dirksen, E. R. (1991). Centriole and basal body formation during ciliogenesis revisited. *Biol. Cell* **72,** 31–38.

Dwyer, N. D., Adler, C. E., Crump, J. G., L'Etoile, N. D., and Bargmann, C. I. (2001). Polarized dendritic transport and the AP-1 mu1 clathrin adaptor UNC-101 localize odorant receptors to olfactory cilia. *Neuron* **31,** 277–287.

Efimenko, E., Bubb, K., Mak, H. Y., Holzman, T., Leroux, M. R., Ruvkun, G., Thomas, J. H., and Swoboda, P. (2005). Analysis of *xbx* genes in *C. elegans*. *Development* **132,** 1923–1934.

Eggenschwiler, J. T., and Anderson, K. V. (2007). Cilia and developmental signaling. *Annu. Rev. Cell Dev. Biol.* **23,** 345–373.

Fan, S., Fogg, V., Wang, Q., Chen, X. W., Liu, C. J., and Margolis, B. (2007). A novel Crumbs3 isoform regulates cell division and ciliogenesis via importin beta interactions. *J. Cell Biol.* **178,** 387–398.

Feistel, K., and Blum, M. (2006). Three types of cilia including a novel 9 + 4 axoneme on the notochordal plate of the rabbit embryo. *Dev. Dyn.* **235,** 3348–3358.

Flannery, R. J., French, D. A., and Kleene, S. J. (2006). Clustering of cyclic-nucleotide-gated channels in olfactory cilia. *Biophys. J.* **91,** 179–188.

Fliegauf, M., Benzing, T., and Omran, H. (2007). When cilia go bad: Cilia defects and ciliopathies. *Nat. Rev. Mol. Cell Biol.* **8,** 880–893.

Follit, J. A., Tuft, R. A., Fogarty, K. E., and Pazour, G. J. (2006). The intraflagellar transport protein IFT20 is associated with the Golgi complex and is required for cilia assembly. *Mol. Biol. Cell* **17,** 3781–3792.

Fujiwara, M., Ishihara, T., and Katsura, I. (1999). A novel WD40 protein, CHE-2, acts cell-autonomously in the formation of *C. elegans* sensory cilia. *Development* **126,** 4839–4848.

Gerdes, J. M., Liu, Y., Zaghloul, N. A., Leitch, C. C., Lawson, S. S., Kato, M., Beachy, P. A., Beales, P. L., DeMartino, G. N., Fisher, S., Badano, J. L., and Katsanis, N. (2007). Disruption of the basal body compromises proteasomal function and perturbs intracellular Wnt response. *Nat. Genet.* **39,** 1350–1360.

Gibbons, B. H., Asai, D. J., Tang, W. J., Hays, T. S., and Gibbons, I. R. (1994). Phylogeny and expression of axonemal and cytoplasmic dynein genes in sea urchins. *Mol. Biol. Cell* **5,** 57–70.

Gilula, N. B., and Satir, P. (1972). The ciliary necklace. A ciliary membrane specialization. *J. Cell Biol.* **53,** 494–509.

Graser, S., Stierhof, Y. D., Lavoie, S. B., Gassner, O. S., Lamla, S., Le Clech, M., and Nigg, E. A. (2007). Cep164, a novel centriole appendage protein required for primary cilium formation. *J. Cell Biol.* **179,** 321–330.

Grissom, P. M., Vaisberg, E. A., and McIntosh, J. R. (2002). Identification of a novel light intermediate chain (D2LIC) for mammalian cytoplasmic dynein 2. *Mol. Biol. Cell* **13,** 817–829.

Han, Y. G., Kwok, B. H., and Kernan, M. J. (2003). Intraflagellar transport is required in *Drosophila* to differentiate sensory cilia but not sperm. *Curr. Biol.* **13,** 1679–1686.

Haycraft, C. J., Swoboda, P., Taulman, P. D., Thomas, J. H., and Yoder, B. K. (2001). The *C. elegans* homolog of the murine cystic kidney disease gene *Tg737* functions in a ciliogenic pathway and is disrupted in *osm-5* mutant worms. *Development* **128,** 1493–1505.

Haycraft, C. J., Schafer, J. C., Zhang, Q., Taulman, P. D., and Yoder, B. K. (2003). Identification of CHE-13, a novel intraflagellar transport protein required for cilia formation. *Exp. Cell Res.* **284,** 251–263.

Haycraft, C. J., Banizs, B., Aydin-Son, Y., Zhang, Q., Michaud, E. J., and Yoder, B. K. (2005). Gli2 and Gli3 localize to cilia and require the intraflagellar transport protein polaris for processing and function. *PLoS Genet.* **1,** e53.

Hirokawa, N., Tanaka, Y., Okada, Y., and Takeda, S. (2006). Nodal flow and the generation of left–right asymmetry. *Cell* **125,** 33–45.

Hong, D. H., Pawlyk, B., Sokolov, M., Strissel, K. J., Yang, J., Tulloch, B., Wright, A. F., Arshavsky, V. Y., and Li, T. (2003). RPGR isoforms in photoreceptor connecting cilia and the transitional zone of motile cilia. *Invest. Ophthalmol. Vis. Sci.* **44,** 2413–2421.

Hou, Y., Pazour, G. J., and Witman, G. B. (2004). A dynein light intermediate chain, D1bLIC, is required for retrograde intraflagellar transport. *Mol. Biol. Cell* **15,** 4382–4394.

Hou, Y., Qin, H., Follit, J. A., Pazour, G. J., Rosenbaum, J. L., and Witman, G. B. (2007). Functional analysis of an individual IFT protein: IFT46 is required for transport of outer dynein arms into flagella. *J. Cell Biol.* **176,** 653–665.

Houde, C., Dickinson, R. J., Houtzager, V. M., Cullum, R., Montpetit, R., Metzler, M., Simpson, E. M., Roy, S., Hayden, M. R., Hoodless, P. A., and Nicholson, D. W. (2006). Hippi is essential for node cilia assembly and Sonic hedgehog signaling. *Dev. Biol.* **300,** 523–533.

Huang, B., Rifkin, M. R., Luck, D. J. L., and Kozler, V. (1977). Temperature-sensitive mutations affecting flagellar assembly and function in *Chlamydomonas reinhardtii*. *J. Cell Biol.* **72,** 67–85.

Huang, K., Diener, D. R., Mitchell, A., Pazour, G. J., Witman, G. B., and Rosenbaum, J. L. (2007). Function and dynamics of PKD2 in *Chlamydomonas reinhardtii* flagella. *J. Cell Biol.* **179,** 501–514.

Huangfu, D., and Anderson, K. V. (2005). Cilia and Hedgehog responsiveness in the mouse. *Proc. Natl Acad. Sci. USA* **102,** 11325–11330.

Huangfu, D., Liu, A., Rakeman, A. S., Murcia, N. S., Niswander, L., and Anderson, K. V. (2003). Hedgehog signalling in the mouse requires intraflagellar transport proteins. *Nature* **426,** 83–87.

Ibanez-Tallon, I., Heintz, N., and Omran, H. (2003). To beat or not to beat: Roles of cilia in development and disease. *Hum. Mol. Genet.* **12,** R27–R35.

Insinna, C., and Besharse, J. C. (2008). Intraflagellar transport and the sensory outer segment of vertebrate photoreceptors. *Dev. Dyn.* **237,** 1982–1992.

Insinna, C., Pathak, N., Perkins, B., Drummond, I., and Besharse, J. C. (2008). The homodimeric kinesin, Kif17, is essential for vertebrate photoreceptor sensory outer segment development. *Dev. Biol.* **316,** 160–170.

Iomini, C., Babaev-Khaimov, V., Sassaroli, M., and Piperno, G. (2001). Protein particles in *Chlamydomonas* flagella undergo a transport cycle consisting of four phases. *J. Cell Biol.* **153,** 13–14.

Ishikawa, H., Kubo, A., and Tsukita, S. (2005). Odf2-deficient mother centrioles lack distal/subdistal appendages and the ability to generate primary cilia. *Nat. Cell Biol.* **7,** 517–524.

Jauregui, A. R., Nguyen, K. C., Hall, D. H., and Barr, M. M. (2008). The *Caenorhabditis elegans* nephrocystins act as global modifiers of cilium structure. *J. Cell Biol.* **180,** 973–988.

Jekely, G., and Arendt, D. (2006). Evolution of intraflagellar transport from coated vesicles and autogenous origin of the eukaryotic cilium. *BioEssays* **28,** 191–198.

Jenkins, P. M., Hurd, T. W., Zhang, L., McEwen, D. P., Brown, R. L., Margolis, B., Verhey, K. J., and Martens, J. R. (2006). Ciliary targeting of olfactory CNG channels requires the CNGB1b subunit and the kinesin-2 motor protein, KIF17. *Curr. Biol.* **16,** 1211–1216.

Jensen, C. G., Poole, C. A., McGlashan, S. R., Marko, M., Issa, Z. I., Vujcich, K. V., and Bowser, S. S. (2004). Ultrastructural, tomographic and confocal imaging of the chondrocyte primary cilium in situ. *Cell Biol. Int.* **28,** 101–110.

Johnson, K. A., and Rosenbaum, J. L. (1992). Polarity of flagellar assembly in *Chlamydomonas*. *J. Cell Biol.* **119,** 1605–1611.

Jurczyk, A., Gromley, A., Redick, S., San Agustin, J., Witman, G., Pazour, G. J., Peters, D. J., and Doxsey, S. (2004). Pericentrin forms a complex with intraflagellar transport proteins and polycystin-2 and is required for primary cilia assembly. *J. Cell Biol.* **166,** 637–643.

Kessel, R., and Kardon, R. (1979). ''Tissues and Organs: A Text-Atlas of Scanning Electron Microscopy.'' WH Freeman and Company, San Francisco, CA.

Khanna, H., Hurd, T. W., Lillo, C., Shu, X., Parapuram, S. K., He, S., Akimoto, M., Wright, A. F., Margolis, B., Williams, D. S., and Swaroop, A. (2005). RPGR-ORF15, which is mutated in retinitis pigmentosa, associates with SMC1, SMC3, and microtubule transport proteins. *J. Biol. Chem.* **280,** 33580–33587.

Kim, J. C., Badano, J. L., Sibold, S., Esmail, M. A., Hill, J., Hoskins, B. E., Leitch, C. C., Venner, K., Ansley, S. J., Ross, A. J., Leroux, M. R., Katsanis, N., *et al.* (2004). The Bardet-Biedl protein BBS4 targets cargo to the pericentriolar region and is required for microtubule anchoring and cell cycle progression. *Nat. Genet.* **36,** 462–470.

King, S. M. (2003). Organization and regulation of the dynein microtubule motor. *Cell Biol. Int.* **27,** 213–215.

Kishimoto, N., Cao, Y., Park, A., and Sun, Z. (2008). Cystic kidney gene seahorse regulates cilia-mediated processes and Wnt pathways. *Dev. Cell* **14,** 954–961.

Kobayashi, T., Gengyo-Ando, K., Ishihara, T., Katsura, I., and Mitani, S. (2007). IFT-81 and IFT-74 are required for intraflagellar transport in *C. elegans*. *Genes Cells* **12,** 593–602.

Kovacs, J. J., Whalen, E. J., Liu, R., Xiao, K., Kim, J., Chen, M., Wang, J., Chen, W., and Lefkowitz, R. J. (2008). Beta-arrestin-mediated localization of smoothened to the primary cilium. *Science* **320,** 1777–1781.

Kozminski, K. G., Johnson, K. A., Forscher, P., and Rosenbaum, J. L. (1993). A motility in the eukaryotic flagellum unrelated to flagellar beating. *Proc. Natl Acad. Sci. USA* **90,** 5519–5523.

Kozminski, K. G., Beech, P. L., and Rosenbaum, J. L. (1995). The *Chlamydomonas* kinesin-like protein FLA10 is involved in motility associated with the flagellar membrane. *J. Cell Biol.* **131,** 1517–1527.

Kunitomo, H., and Iino, Y. (2008). *Caenorhabditis elegans* DYF-11, an orthologue of mammalian Traf3ip1/MIP-T3, is required for sensory cilia formation. *Genes Cells* **13,** 13–25.

Lansbergen, G., and Akhmanova, A. (2006). Microtubule plus end: A hub of cellular activities. *Traffic* **7,** 499–507.

Lehman, J. M., Michaud, E. J., Schoeb, T. R., Aydin-Son, Y., Miller, M., and Yoder, B. K. (2008). The Oak Ridge Polycystic Kidney mouse: Modeling ciliopathies of mice and men. *Dev. Dyn.* **237,** 1960–1971.

Leroux, M. R. (2007). Taking vesicular transport to the cilium. *Cell* **129,** 1041–1043.

Li, J. B., Gerdes, J. M., Haycraft, C. J., Fan, Y., Teslovich, T. M., May-Simera, H., Li, H., Blacque, O. E., Li, L., Leitch, C. C., Lewis, R. A., Green, J. S., *et al.* (2004). Comparative genomics identifies a flagellar and basal body proteome that includes the BBS5 human disease gene. *Cell* **117,** 541–552.

Li, C., Inglis, P. N., Leitch, C. C., Efimenko, E., Zaghloul, N. A., Mok, C. A., Davis, E. E., Bialas, N. J., Healey, M. P., Heon, E., Zhen, M., Swoboda, P., *et al.* (2008). An essential role for DYF-11/MIP-T3 in assembling functional intraflagellar transport complexes. *PLoS Genet.* **4,** e1000044.

Low, S. H., Vasanth, S., Larson, C. H., Mukherjee, S., Sharma, N., Kinter, M. T., Kane, M. E., Obara, T., and Weimbs, T. (2006). Polycystin-1, STAT6, and P100 function in a pathway that transduces ciliary mechanosensation and is activated in polycystic kidney disease. *Dev. Cell* **10,** 57–69.

Lucker, B. F., Behal, R. H., Qin, H., Siron, L. C., Taggart, W. D., Rosenbaum, J. L., and Cole, D. G. (2005). Characterization of the intraflagellar transport complex B core: Direct interaction of the IFT81 and IFT74/72 subunits. *J. Biol. Chem.* **280,** 27688–27696.

Marshall, W. F. (2008). The cell biological basis of ciliary disease. *J. Cell Biol.* **180,** 17–21.

Marshall, W. F., and Rosenbaum, J. L. (2001). Intraflagellar transport balances continuous turnover of outer doublet microtubules: Implications for flagellar length control. *J. Cell Biol.* **155,** 405–414.

Marszalek, J. R., Ruiz-Lozano, P., Roberts, E., Chien, K. R., and Goldstein, L. S. (1999). Situs inversus and embryonic ciliary morphogenesis defects in mouse mutants lacking the KIF3A subunit of kinesin-II. *Proc. Natl Acad. Sci. USA* **96,** 5043–5048.

May, S. R., Ashique, A. M., Karlen, M., Wang, B., Shen, Y., Zarbalis, K., Reiter, J., Ericson, J., and Peterson, A. S. (2005). Loss of the retrograde motor for IFT disrupts localization of Smo to cilia and prevents the expression of both activator and repressor functions of Gli. *Dev. Biol.* **287,** 378–389.

Merchant, S. S., Prochnik, S. E., Vallon, O., Harris, E. H., Karpowicz, S. J., Witman, G. B., Terry, A., Salamov, A., Fritz-Laylin, L. K., Marechal-Drouard, L., Marshall, W. F., Qu, L. H., *et al.* (2007). The *Chlamydomonas* genome reveals the evolution of key animal and plant functions. *Science* **318,** 245–250.

Mikami, A., Tynan, S. H., Hama, T., Luby-Phelps, K., Saito, T., Crandall, J. E., Besharse, J. C., and Vallee, R. B. (2002). Molecular structure of cytoplasmic dynein 2 and its distribution in neuronal and ciliated cells. *J. Cell Sci.* **115,** 4801–4808.

Miller, M. S., Esparza, J. M., Lippa, A. M., Lux, F. G., III, Cole, D. G., and Dutcher, S. K. (2005). Mutant kinesin-2 motor subunits increase chromosome loss. *Mol. Biol. Cell* **16,** 3810–3820.

Mitchell, B. F., Pedersen, L. B., Feely, M., Rosenbaum, J. L., and Mitchell, D. R. (2005). ATP production in *Chlamydomonas reinhardtii* flagella by glycolytic enzymes. *Mol. Biol. Cell* **16,** 4509–4518.

Moritz, O. L., Tam, B. M., Hurd, L. L., Peranen, J., Deretic, D., and Papermaster, D. S. (2001). Mutant rab8 Impairs docking and fusion of rhodopsin-bearing post-Golgi membranes and causes cell death of transgenic Xenopus rods. *Mol. Biol. Cell* **12,** 2341–2351.

Morris, R. L., and Scholey, J. M. (1997). Heterotrimeric kinesin-II is required for the assembly of motile 9 + 2 ciliary axonemes on sea urchin embryos. *J. Cell Biol.* **138,** 1009–1022.

Mrug, M., Li, R., Cui, X., Schoeb, T. R., Churchill, G. A., and Guay-Woodford, L. M. (2005). Kinesin family member 12 is a candidate polycystic kidney disease modifier in the cpk mouse. *J. Am. Soc. Nephrol.* **16,** 905–916.

Mueller, J., Perrone, C. A., Bower, R., Cole, D. G., and Porter, M. E. (2005). The *FLA3* KAP subunit is required for localization of kinesin-2 to the site of flagellar assembly and processive anterograde intraflagellar transport. *Mol. Biol. Cell* **16,** 1341–1354.

Mukhopadhyay, S., Lu, Y., Shaham, S., and Sengupta, P. (2008). Sensory signaling-dependent remodeling of olfactory cilia architecture in *C. elegans*. *Dev. Cell* **14,** 762–774.

Murayama, T., Toh, Y., Ohshima, Y., and Koga, M. (2005). The *dyf-3* gene encodes a novel protein required for sensory cilium formation in *Caenorhabditis elegans*. *J. Mol. Biol.* **346,** 677–687.

Nachury, M. V., Loktev, A. V., Zhang, Q., Westlake, C. J., Peranen, J., Merdes, A., Slusarski, D. C., Scheller, R. H., Bazan, J. F., Sheffield, V. C., and Jackson, P. K. (2007). A core complex of BBS proteins cooperates with the GTPase Rab8 to promote ciliary membrane biogenesis. *Cell* **129,** 1201–1213.

Nair, S., Guerra, C., and Satir, P. (1999). A Sec7-related protein in *Paramecium*. *FASEB J.* **13,** 1249–1257.

Nakagawa, T., Setou, M., Seog, D., Ogasawara, K., Dohmae, N., Takio, K., and Hirokawa, N. (2000). A novel motor, KIF13A, transports mannose-6-phosphate receptor to plasma membrane through direct interaction with AP-1 complex. *Cell* **103,** 569–581.

Nonaka, S., Tanaka, Y., Okada, Y., Takeda, S., Harada, A., Kanai, Y., Kido, M., and Hirokawa, N. (1998). Randomization of left–right asymmetry due to loss of nodal cilia generating leftward flow of extraembryonic fluid in mice lacking KIF3B motor protein. *Cell* **95,** 829–837.

Omori, Y., Zhao, C., Saras, A., Mukhopadhyay, S., Kim, W., Furukawa, T., Sengupta, P., Veraksa, A., and Malicki, J. (2008). Elipsa is an early determinant of ciliogenesis that links the IFT particle to membrane-associated small GTPase Rab8. *Nat. Cell Biol.* **10,** 437–444.

Orozco, J. T., Wedaman, K. P., Signor, D., Brown, M., Rose, L., and Scholey, J. M. (1999). Movement of motor and cargo along cilia. *Nature* **398,** 674.

Ou, G., Blacque, O. E., Snow, J. J., Leroux, M. R., and Scholey, J. M. (2005a). Functional coordination of intraflagellar transport motors. *Nature* **436,** 583–587.

Ou, G., Qin, H., Rosenbaum, J. L., and Scholey, J. M. (2005b). The PKD protein qilin undergoes intraflagellar transport. *Curr. Biol.* **15,** R410–R411.

Paintrand, M., Moudjou, M., Delacroix, H., and Bornens, M. (1992). Centrosome organization and centriole architecture: Their sensitivity to divalent cations. *J. Struct. Biol.* **108,** 107–128.

Pan, J., and Snell, W. J. (2005). *Chlamydomonas* shortens its flagella by activating axonemal disassembly, stimulating IFT particle trafficking, and blocking anterograde cargo loading. *Dev. Cell* **9,** 431–438.

Pan, J., and Snell, W. (2007). The primary cilium: Keeper of the key to cell division. *Cell* **129,** 1255–1257.

Pan, J., Wang, Q., and Snell, W. J. (2005). Cilium-generated signaling and cilia-related disorders. *Lab. Invest.* **85,** 452–463.

Pan, X., Ou, G., Civelekoglu-Scholey, G., Blacque, O. E., Endres, N. F., Tao, L., Mogilner, A., Leroux, M. R., Vale, R. D., and Scholey, J. M. (2006). Mechanism of transport of IFT particles in *C. elegans* cilia by the concerted action of kinesin-II and OSM-3 motors. *J. Cell Biol.* **174,** 1035–1045.

Park, T. J., Mitchell, B. J., Abitua, P. B., Kintner, C., and Wallingford, J. B. (2008). Dishevelled controls apical docking and planar polarization of basal bodies in ciliated epithelial cells. *Nat. Genet.* **40,** 871–879.

Pathak, N., Obara, T., Mangos, S., Liu, Y., and Drummond, I. A. (2007). The zebrafish *fleer* gene encodes an essential regulator of cilia tubulin polyglutamylation. *Mol. Biol. Cell* **18,** 4353–4364.

Pazour, G. J., and Rosenbaum, J. L. (2002). Intraflagellar transport and cilia-dependent diseases. *Trends Cell Biol.* **12,** 551–555.

Pazour, G. J., and Witman, G. B. (2003). The vertebrate primary cilium is a sensory organelle. *Curr. Opin. Cell Biol.* **15,** 105–110.

Pazour, G. J., Wilkerson, C. G., and Witman, G. B. (1998). A dynein light chain is essential for retrograde particle movement in intraflagellar transport (IFT). *J. Cell Biol.* **141,** 979–992.

Pazour, G. J., Dickert, B. L., and Witman, G. B. (1999). The DHC1b (DHC2) isoform of cytoplasmic dynein is required for flagellar assembly. *J. Cell Biol.* **144,** 473–481.

Pazour, G. J., Dickert, B. L., Vucica, Y., Seeley, E. S., Rosenbaum, J. L., Witman, G. B., and Cole, D. G. (2000). *Chlamydomonas* IFT88 and its mouse homologue, polycystic kidney disease gene *tg737*, are required for assembly of cilia and flagella. *J. Cell Biol.* **151,** 709–718.

Pazour, G. J., San Agustin, J. T., Follit, J. A., Rosenbaum, J. L., and Witman, G. B. (2002). Polycystin-2 localizes to kidney cilia and the ciliary level is elevated in orpk mice with polycystic kidney disease. *Curr. Biol.* **12,** R378–R380.

Pazour, G. J., Agrin, N., Leszyk, J., and Witman, G. B. (2005). Proteomic analysis of a eukaryotic cilium. *J. Cell Biol.* **170,** 103–113.

Peden, E. M., and Barr, M. M. (2005). The KLP-6 kinesin is required for male mating behaviors and polycystin localization in *Caenorhabditis elegans*. *Curr. Biol.* **15,** 394–404.

Pedersen, L. B., Geimer, S., Sloboda, R. D., and Rosenbaum, J. L. (2003). The microtubule plus end-tracking protein EB1 is localized to the flagellar tip and basal bodies in *Chlamydomonas reinhardtii*. *Curr. Biol.* **13,** 1969–1974.

Pedersen, L. B., Miller, M. S., Geimer, S., Leitch, J. M., Rosenbaum, J. L., and Cole, D. G. (2005). *Chlamydomonas* IFT172 is encoded by *FLA11*, interacts with CrEB1, and regulates IFT at the flagellar tip. *Curr. Biol.* **15,** 262–266.

Pedersen, L. B., Geimer, S., and Rosenbaum, J. L. (2006). Dissecting the molecular mechanisms of intraflagellar transport in *Chlamydomonas*. *Curr. Biol.* **16,** 450–459.

Pedersen, L. B., Veland, I. R., Schroder, J. M., and Christensen, S. T. (2008). Assembly of primary cilia. *Dev. Dyn.* **237,** 1993–2006.

Perkins, L. A., Hedgecock, E. M., Thomson, J. N., and Culotti, J. G. (1986). Mutant sensory cilia in the nematode *Caenorhabditis elegans*. *Dev. Biol.* **117,** 456–487.

Perrone, C. A., Tritschler, D., Taulman, P., Bower, R., Yoder, B. K., and Porter, M. E. (2003). A novel dynein light intermediate chain colocalizes with the retrograde motor for intraflagellar transport at sites of axoneme assembly in *Chlamydomonas* and mammalian cells. *Mol. Biol. Cell* **14,** 2041–2056.

Pfister, K. K., Fisher, E. M., Gibbons, I. R., Hays, T. S., Holzbaur, E. L., McIntosh, J. R., Porter, M. E., Schroer, T. A., Vaughan, K. T., Witman, G. B., King, S. M., and Vallee, R. B. (2005). Cytoplasmic dynein nomenclature. *J. Cell Biol.* **171,** 411–413.

Piperno, G., and Mead, K. (1997). Transport of a novel complex in the cytoplasmic matrix of *Chlamydomonas* flagella. *Proc. Natl Acad. Sci. USA* **94,** 4457–4462.

Piperno, G., Siuda, E., Henderson, S., Segil, M., Vaananen, H., and Sassaroli, M. (1998). Distinct mutants of retrograde intraflagellar transport (IFT) share similar morphological and molecular defects. *J. Cell Biol.* **143,** 1591–1601.

Plotnikova, O. V., Golemis, E. A., and Pugacheva, E. N. (2008). Cell cycle-dependent ciliogenesis and cancer. *Cancer Res.* **68,** 2058–2061.

Porter, M. E., Bower, R., Knott, J. A., Byrd, P., and Dentler, W. (1999). Cytoplasmic dynein heavy chain 1b is required for flagellar assembly in *Chlamydomonas*. *Mol. Biol. Cell* **10,** 693–712.

Qin, H., Rosenbaum, J. L., and Barr, M. M. (2001). An autosomal recessive polycystic kidney disease gene homolog is involved in intraflagellar transport in *C. elegans* ciliated sensory neurons. *Curr. Biol.* **11,** 457–461.

Qin, H., Diener, D. R., Geimer, S., Cole, D. G., and Rosenbaum, J. L. (2004). Intraflagellar transport (IFT) cargo: IFT transports flagellar precursors to the tip and turnover products to the cell body. *J. Cell Biol.* **164,** 255–266.

Qin, H., Burnette, D., Bae, Y. K., Forscher, P., Barr, M. M., and Rosenbaum, J. L. (2005). Intraflagellar transport is required for the vectorial movement of TRPV channels in the ciliary membrane. *Curr. Biol.* **15,** 1695–1699.

Qin, H., Wang, Z., Diener, D., and Rosenbaum, J. (2007). Intraflagellar transport protein 27 is a small G protein involved in cell-cycle control. *Curr. Biol.* **17,** 193–202.

Quarmby, L. M., and Parker, J. D. (2005). Cilia and the cell cycle? *J. Cell Biol.* **169,** 707–710.

Rana, A. A., Barbera, J. P., Rodriguez, T. A., Lynch, D., Hirst, E., Smith, J. C., and Beddington, R. S. (2004). Targeted deletion of the novel cytoplasmic dynein mD2LIC disrupts the embryonic organiser, formation of the body axes and specification of ventral cell fates. *Development* **131,** 4999–5007.

Reese, T. S. (1965). Olfactory cilia in the frog. *J. Cell Biol.* **25,** 209–230.

Reiter, J. F., and Mostov, K. (2006). Vesicle transport, cilium formation, and membrane specialization: The origins of a sensory organelle. *Proc. Natl Acad. Sci. USA* **103,** 18383–18384.

Rohatgi, R., Milenkovic, L., and Scott, M. P. (2007). Patched1 regulates hedgehog signaling at the primary cilium. *Science* **317,** 372–376.

Rompolas, P., Pedersen, L. B., Patel-King, R. S., and King, S. M. (2007). *Chlamydomonas* FAP133 is a dynein intermediate chain associated with the retrograde intraflagellar transport motor. *J. Cell Sci.* **120,** 3653–3665.

Rosenbaum, J. L., and Witman, G. B. (2002). Intraflagellar transport. *Nat. Rev. Mol. Cell Biol.* **3,** 813–825.

Salomon, R., Saunier, S., and Niaudet, P. (2008). Nephronophthisis. Pediatr. Nephrol. [Epub ahead of print].

Santos, N., and Reiter, J. F. (2008). Building it up and taking it down: The regulation of vertebrate ciliogenesis. *Dev. Dyn.* **237,** 1972–1981.

Sarpal, R., Todi, S. V., Sivan-Loukianova, E., Shirolikar, S., Subramanian, N., Raff, E. C., Erickson, J. W., Ray, K., and Eberl, D. F. (2003). *Drosophila* KAP interacts with the kinesin II motor subunit KLP64D to assemble chordotonal sensory cilia, but not sperm tails. *Curr. Biol.* **13,** 1687–1696.

Satir, P., and Christensen, S. T. (2006). Overview of structure and function of mammalian cilia. *Annu. Rev. Physiol.* **69,** 377–400.

Schafer, J. C., Haycraft, C. J., Thomas, J. H., Yoder, B. K., and Swoboda, P. (2003). *XBX-1* encodes a dynein light intermediate chain required for retrograde intraflagellar transport and cilia assembly in *Caenorhabditis elegans*. *Mol. Biol. Cell* **14,** 2057–2070.

Schmitt, A., and Wolfrum, U. (2001). Identification of novel molecular components of the photoreceptor connecting cilium by immunoscreens. *Exp. Eye Res.* **73,** 837–849.

Schneider, L., Clement, C. A., Teilmann, S. C., Pazour, G. P., Hoffmann, E. K., Satir, P., and Christensen, S. T. (2005). PDGFRαα signaling is regulated through the primary cilium in fibroblasts. *Curr. Biol.* **15,** 1861–1866.

Scholey, J. M. (2003). Intraflagellar transport. *Annu. Rev. Cell Dev. Biol.* **19,** 423–443.

Scholey, J. M. (2008). Intraflagellar transport motors in cilia: Moving along the cell's antenna. *J. Cell Biol.* **180,** 23–29.

Scholey, J. M., and Anderson, K. V. (2006). Intraflagellar transport and cilium-based signaling. *Cell* **125,** 439–442.

Schrøder, J. M., Schneider, L., Christensen, S. T., and Pedersen, L. B. (2007). EB1 is required for primary cilia assembly in fibroblasts. *Curr. Biol.* **17,** 1134–1139.

Shakir, M. A., Fukushige, T., Yasuda, H., Miwa, J., and Siddiqui, S. S. (1993). *C. elegans osm-3* gene mediating osmotic avoidance behaviour encodes a kinesin-like protein. *NeuroReport* **4,** 891–894.

Shalom, O., Shalva, N., Altschuler, Y., and Motro, B. (2008). The mammalian Nek1 kinase is involved in primary cilium formation. *FEBS Lett.* **582,** 1465–1470.

Signor, D., Wedaman, K. P., Orozco, J. T., Dwyer, N. D., Bargmann, C. I., Rose, L. S., and Scholey, J. M. (1999a). Role of a class DHC1b dynein in retrograde transport of IFT motors and IFT raft particles along cilia, but not dendrites, in chemosensory neurons of living *Caenorhabditis elegans*. *J. Cell Biol.* **147,** 519–530.

Signor, D., Wedaman, K. P., Rose, L. S., and Scholey, J. M. (1999b). Two heteromeric kinesin complexes in chemosensory neurons and sensory cilia of *Caenorhabditis elegans*. *Mol. Biol. Cell* **10,** 345–360.

Singla, V., and Reiter, J. F. (2006). The primary cilium as the cell's antenna: Signaling at a sensory organelle. *Science* **313,** 629–633.

Sloboda, R. D. (2002). A healthy understanding of intraflagellar transport. *Cell Motil. Cytoskeleton* **52,** 1–8.

Sloboda, R. D., and Howard, L. (2007). Localization of EB1, IFT polypeptides, and kinesin-2 in *Chlamydomonas* flagellar axonemes via immunogold scanning electron microscopy. *Cell Motil. Cytoskeleton* **64,** 446–460.

Snow, J. J., Ou, G., Gunnarson, A. L., Walker, M. R. S., Zhou, H. M., Brust-Mascher, I., and Scholey, J. M. (2004). Two anterograde intraflagellar transport motors cooperate to build sensory cilia on *C. elegans* neurons. *Nat. Cell Biol.* **6,** 1109–1113.

Sorokin, S. (1962). Centrioles and the formation of rudimentary cilia by fibroblasts and smooth muscle cells. *J. Cell Biol.* **15,** 363–377.

Stolc, V., Samata, M. P., Tongprasit, W., and Marshall, W. F. (2005). Genome-wide transcriptional analysis of flagellar regeneration in *Chlamydomonas reinhardtii* identifies orthologs of ciliary disease genes. *Proc. Natl Acad. Sci. USA* **102,** 3703–3707.

Sun, Z., Amsterdam, A., Pazour, G. J., Cole, D. G., Miller, M. S., and Hopkins, N. (2004). A genetic screen identifies cilia genes as a principal cause of cystic kidney. *Development* **131,** 4085–4093.

Surpili, M. J., Delben, T. M., and Kobarg, J. (2003). Identification of proteins that interact with the central coiled-coil region of the human protein kinase NEK1. *Biochemistry* **42,** 15369–15376.

Takeda, S., Yonekawa, Y., Tanaka, Y., Okada, Y., Nonaka, S., and Hirokawa, N. (1999). Left–right asymmetry and kinesin superfamily protein KIF3A: New insights in determination of laterality and mesoderm induction by kif3A−/− mice analysis. *J. Cell Biol.* **145,** 825–836.

Tallila, J., Jakkula, E., Peltonen, L., Salonen, R., and Kestila, M. (2008). Identification of CC2D2A as a Meckel syndrome gene adds an important piece to the ciliopathy puzzle. *Am. J. Hum. Genet.* **82,** 1361–1367.

Tan, P. L., Barr, T., Inglis, P. N., Mitsuma, N., Huang, S. M., Garcia-Gonzalez, M. A., Bradley, B. A., Coforio, S., Albrecht, P. J., Watnick, T., Germino, G. G., Beales, P. L., *et al.* (2007). Loss of Bardet-Biedl syndrome proteins causes defects in peripheral sensory innervation and function. *Proc. Natl Acad. Sci. USA* **104,** 17524–17529.

Tobin, J. L., and Beales, P. L. (2007). Bardet-Biedl syndrome: Beyond the cilium. *Pediatr. Nephrol.* **22,** 926–936.

Town, T., Breunig, J. J., Sarkisian, M. R., Spilianakis, C., Ayoub, A. E., Liu, X., Ferrandino, A. F., Gallagher, A. R., Li, M. O., Rakic, P., and Flavell, R. A. (2008). The stumpy gene is required for mammalian ciliogenesis. *Proc. Natl Acad. Sci. USA* **105,** 2853–2858.

Tran, P. V., Haycraft, C. J., Besschetnova, T. Y., Turbe-Doan, A., Stottmann, R. W., Herron, B. J., Chesebro, A. L., Qiu, H., Scherz, P. J., Shah, J. V., Yoder, B. K., and Beier, D. R. (2008). THM1 negatively modulates mouse sonic hedgehog signal transduction and affects retrograde intraflagellar transport in cilia. *Nat. Genet.* **40,** 403–410.

Tsao, C. C., and Gorovsky, M. A. (2008a). Different effects of *Tetrahymena* IFT172 domains on anterograde and retrograde intraflagellar transport. *Mol. Biol. Cell* **19,** 1450–1461.

Tsao, C. C., and Gorovsky, M. A. (2008b). *Tetrahymena* IFT122A is not essential for cilia assembly but plays a role in returning IFT proteins from the ciliary tip to the cell body. *J. Cell Sci.* **121,** 428–436.

Tsujikawa, M., and Malicki, J. (2004). Intraflagellar transport genes are essential for differentiation and survival of vertebrate sensory neurons. *Neuron* **42,** 703–716.

Vieira, O. V., Gaus, K., Verkade, P., Fullekrug, J., Vaz, W. L., and Simons, K. (2006). FAPP2, cilium formation, and compartmentalization of the apical membrane in polarized Madin-Darby canine kidney (MDCK) cells. *Proc. Natl Acad. Sci. USA* **103,** 18556–18561.

von Schnakenburg, C., Fliegauf, M., and Omran, H. (2007). Nephrocystin and ciliary defects not only in the kidney? *Pediatr. Nephrol.* **22,** 765–769.

Walther, Z., Vashishtha, M., and Hall, J. L. (1994). The *Chlamydomonas FLA10* gene encodes a novel kinesin-homologous protein. *J. Cell Biol.* **126,** 175–188.

Wang, Q., Pan, J., and Snell, W. J. (2006). Intraflagellar transport particles participate directly in cilium-generated signaling in *Chlamydomonas*. *Cell* **125,** 549–562.

White, M. C., and Quarmby, L. M. (2008). The NIMA-family kinase, Nek1 affects the stability of centrosomes and ciliogenesis. *BMC Cell Biol.* **9,** 29.

Wickstead, B., and Gull, K. (2006). A ''holistic'' kinesin phylogeny reveals new kinesin families and predicts protein functions. *Mol. Biol. Cell* **17,** 1734–1743.

Williams, C. L., Winkelbauer, M. E., Schafer, J. C., Michaud, E. J., and Yoder, B. K. (2008). Functional redundancy of the B9 proteins and nephrocystins in *Caenorhabditis elegans* ciliogenesis. *Mol. Biol. Cell* **19,** 2154–2168.

Wilson, N. F., and Lefebvre, P. A. (2004). Regulation of flagellar assembly by glycogen synthase kinase 3 in *Chlamydomonas reinhardtii*. *Eukaryot. Cell* **3,** 1307–1319.

Witman, G. B., Carlson, L., Berliner, J., and Rosenbaum, J. L. (1972). *Chlamydomonas* flagella. I. Isolation and electrophoretic analysis of microtubules, matrix, membranes, and mastigonemes. *J. Cell Biol.* **54,** 507–539.

Yoder, B. K., Hou, X., and Guay-Woodford, L. M. (2002). The polycystic kidney disease proteins, polycystin-1, polycystin-2, polaris, and cystin, are co-localized in renal cilia. *J. Am. Soc. Nephrol.* **13,** 2508–2516.

Yoshimura, S., Egerer, J., Fuchs, E., Haas, A. K., and Barr, F. A. (2007). Functional dissection of Rab GTPases involved in primary cilium formation. *J. Cell Biol.* **178,** 363–369.